1970

The hematology of hog cholera

Delmar Ronald Cassidy
Iowa State University

THE HEMATOLOGY OF HOG CHOLERA

by

Delmar Ronald Cassidy

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Veterinary Pathology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

Head of Major Department

Signature was redacted for privacy.

Dean of Graduate College

Iowa State University
Of Science and Technology
Ames, Iowa

1970

PLEASE NOTE:

Several pages contain colored illustrations. Filmed in the best possible way.

UNIVERSITY MICROFILMS

TABLE OF CONTENTS

INTRODUCTION

Hog cholera is a common disease of swine, affecting pigs of all ages, breeds and sexes. The disease is infectious, highly contagious, and generally fatal. It is characterized clinically by anorexia, fever, and extreme debility. Lesions commonly observed at necropsy include petechial and ecchymotic hemorrhages, hyperemia and infarction. Hog cholera was apparently first recognized as an important disease around 1830 in the states of Ohio and Indiana. In 1903 a virus was shown to be the etiologic agent of hog cholera. Recently, numerous outbreaks of the disease have been caused by viruses of low and moderate virulence.

Leukopenia has been considered one of the most reliable criteria available for the diagnosis of hog cholera during the past 50 years. However, the exact cause of the reduction of white blood cell numbers has remained a mystery. Anemia in varying degrees of severity has also been described during field outbreaks of hog cholera and no explanation of the mechanism producing this change is known. Even though anemia and leukopenia are salient questions in the practical problem of diagnosing hog cholera, there has been little study on the basic pathogenetic mechanism of hog cholera in the swine hemopoietic organs.

It was the purpose of this research to investigate the pathogenesis of hog cholera in the hemopoietic organs of pigs

infected with 2 strains of hog cholera virus. This was an attempt to understand the fundamental mechanisms responsible for the changes produced by hog cholera in the peripheral blood of infected animals. It is believed that knowledge from this study will contribute to a more complete understanding of the mechanism of this disease of swine.

REVIEW OF THE LITERATURE

Early History of Hog Cholera

Hog cholera (HC) was unknown 150 years ago, yet no other disease of swine has produced such a high mortality or has been so dreaded. Losses to the American livestock industry from this disease during the period from 1914 to 1924 were estimated at 415 million dollars (Atherton, 1923). In 1950 the annual loss was placed at between 30 and 40 million dollars (Quin, 1950). More recently Mulhern (1961) appraised the average yearly cost of the disease at between 40 to 60 million dollars.

In this review only the hematologic and some related changes observed in the blood and other tissues of swine infected with HC virus (HCV) will be considered. For a more comprehensive review and discussion the work of Dunne (1964a) should be consulted.

Hanson (1957) published a critical review of the earliest reports of HC in the United States. He concluded from these reports that HC was first recognized in an area which can be encompassed by a circle with a radius of 200 miles, having its center in Clay County, Tennessee. This includes most of Kentucky, Tennessee, areas of Indiana, Alabama, Georgia and North Carolina. Hanson further concluded that the acute disease was recognized in Kentucky, Tennessee and Indiana during the early 1840's, but the virus may have existed in

swine of that area as an atypical disease for many years. Kernkamp (1961) also reviewed early reports of HC in the United States and summarized early research work on the disease.

Most early workers attributed the disease to an infectious agent but were less certain as to the exact nature of the causative agent. Salmon and Smith (1886) carried out extensive experiments in an effort to determine the etiology of HC. During these investigations Salmon isolated a bacterium, the genus of which was later named for him, which he considered to be the cause of the disease. It remained for DeSchweinitz and Dorset (1903) to discover that a virus was the cause of HC. The discovery of the viral etiology of HC established an unequivocal basis for the diagnosis of the disease. However the impracticality of withholding a diagnosis until proof of the presence of the virus in filtered blood or other tissue had been confirmed by inoculation of susceptible swine, made it imperative that other diagnostic criteria be developed.

Early Hematologic Studies

During a study of the effect of HCV on the cellular morphology of horse and pig blood, King and Wilson (1910) observed a number of interesting changes. No significant effect of the virus on horse blood was apparent. In the blood of HC infected pigs the number of erythrocytes and amount of

hemoglobin were decreased. The anemic condition became increasingly severe as the disease progressed. When compared with blood from normal swine, blood from HC infected pigs was leukopenic, with an average decrease of 5,000 leukocytes per cu. mm. A relative reduction of some types of leukocytes as shown by differential white blood cell (WBC) counts involved a decrease of 4 percent of the lymphocytes, 4 percent of the polymorphonuclear neutrophils and 0.1 percent of the polymorphonuclear eosinophils. They reported an average increase of 4.8 percent of the large mononuclear cells, 2.8 percent of the basophils and 0.8 percent of the transitional forms.

Lewis et al. (1914) reported a reduction in red and white cell numbers and hemoglobin values in the blood of swine infected with HCV. They mentioned that not all cases manifested these changes to the same extent but there was a general tendency toward a reduction in total red blood cell (TRBC) number and the amount of hemoglobin present. A lowering of the total white blood count (TWBC) numbers also occurred; however, this change was not as uniformly present as the two aforementioned changes. These workers described 2 forms of HC, an acute disease which produced death within a few hours to a few days and a chronic form which was a much less severe form of the disease. The severity of the infection was reflected in the degree of depression of the red and white blood cell numbers and hemoglobin values. Analysis of urine from infected pigs

failed to detect any significant deviations from normal values.

Dinwiddie (1914) reported the results of an intensive study of the morphology of the blood cells from 18 normal and 24 HCV infected pigs. After carefully reviewing the description of the red blood cell (RBC) inclusions observed in blood from some of the infected animals, it seems probable to me that some of the animals were concurrently infected with porcine eperythrozoonosis. The report lists the following as normal values for the 18 normal young swine. Total RBC numbers averaged between 7 to 9 million per cu. mm., while TWBC numbers averaged between 7 to 12 thousand per cu. mm. The presence of 2 types of lymphocytes in the swine blood was also reported. Difficulties were encountered in attempting to classify the WBC's from HCV infected animals on their morphologic characteristics. The most obvious changes detected in the blood of the HCV infected pigs were demonstrable at the onset of the disease. He further remarked that the leukopenia was so pronounced that one could readily observe the scarcity of leukocytes when examining a stained blood smear even before a count was made. In some cases a decrease of mature but increase of immature neutrophils occurred. Other infected animals manifested a marked polymorphoneuclear leukocytosis which was due to some inflammatory complication which most commonly was pleurisy or pneumonia. An absence of uniformity in the changes in the differential WBC count was also discussed. A moderately rapid diminution in TRBC numbers was

also described but was not considered to be an outstanding feature of the disease. Immature erythrocytes generally increased in numbers after infection, particularly if the case of HC was of long duration.

In Germany, Regner (1923) attempted to differentiate hog cholera, swine plague and swine erysipelas by means of the blood picture produced in each disease. He described a rapidly occurring leukopenia in acute hog cholera with a relative lymphocytosis of about 75 percent but no change in RBC numbers. Eosinophils were absent from the smears of peripheral blood. Erysipelas produced a marked increase in nucleated red blood cells, a leukocytosis and a normal neutrophil-lymphocyte ratio. Cases of chronic hog cholera and mixed infections of swine plague and hog cholera, manifested leukopenia and a relative increase in lymphocytes. Pure infections of swine plague (bronchopneumonia) resulted in a leukocytosis with a relative neutrophilia. Eosinophils and basophils were absent from peripheral blood smears collected from cases of both diseases.

Lewis and Shope (1929a, 1929b) demonstrated in their study of blood from swine infected with HCV that the route of inoculation and amount of virus injected did not materially affect the resulting blood cell alterations. All 6 pigs studied showed a moderate, progressive anemia. Attempts by the hemopoietic systems of the animals to compensate for the anemia were detected by the showers of nucleated RBC's

in the peripheral blood during the early stages of the disease. A profound leukopenia was evident in all of the animals and most pronounced in 5 of the 6 animals as early as 48 hours after infection. The leukopenia in these 5 animals preceded any clinical manifestation of the disease, including a rise in temperature. By the time the temperature elevation did occur, TWBC counts ranging from 2,000 to 4,000 per cu. mm. were recorded. If the animals lived long enough, these extreme leukopenias persisted from 8-13 days at which time the leukocyte counts rose to somewhat higher levels. At no time did the TWBC counts reach normal levels. Study of stained blood films indicated that the polymorphonuclear neutrophilic leukocytes were the most severely affected, however cells of the lymphocytic series were also involved. These same workers, using blood supravitally stained with neutral red and Janus green, demonstrated that differential counts on fixed blood smears do not truly represent the actual hematologic picture. Blood which showed no polymorphonuclear neutrophils and eosinophils in fixed preparations, when supravitally stained, often contained these cell types which were very badly damaged, much vaculoated and apparently very fragile. Blood from early cases of HC, when incubated for 7 hours at 37 C with heparin as the anticoagulant, was characterized by a further reduction in TWBC numbers. Blood changes in swine infected by HCV from 5 different farm herds were also studied. The results confirmed that the experimentally induced and natural-

ly occurring disease produced a leukopenia which was virtually similar in all characteristics studied. The effect on the WBC count in pigs was studied in hemorrhagic septicemia, acute infectious enteritis, swine influenza, porcine ascariasis and a posterior paralysis of unknown cause. All of the diseases, with the exception of swine influenza, produced a leukocytosis of varying degree and duration. In pigs infected with swine influenza, a slight to moderate leukopenia was evident. In only 2 of the 25 cases of swine influenza, were the counts low enough to have been confused with HC. The authors concluded that a leukocyte count of 8,000 WBC's per cu. mm. or less in 3 sick pigs in a herd are reliable indicators that the condition in the herd is HC.

Specific Hematologic Examinations

Total white blood cell count

The leukocyte response in pigs immunized by the virus and serum, or the simultaneous method of HC immunization, has been the subject of several studies. Lewis and Shope (1929a) stated that the so-called double treatment to hogs does not produce a leukopenia; however, several reports presenting conflicting data have been published. Cahill (1929) reported that 2 healthy pigs given the simultaneous treatment had TWBC counts of 7,300 and 4,400 per cu. mm. the following day. After 24 hours the decrease stopped and a gradual leukocytosis occurred until the eighth day, when both animals had counts

of 14,000 WBC per cu. mm. Cole (1932) studied 28 simultaneously treated pigs and observed a distinct, although transitory, leukopenia following the treatment. Leukopenia was followed, in many cases, by a leukocytosis. The severity of the leukopenia was to a certain extent related to the dose of serum, the leukopenia being more severe in the animals receiving the smaller doses of serum. A transitory leukopenia following vaccination with lapinized HCV vaccines without anti-HCV serum has been observed and reported by Weide and Twiehaus (1959). Workers in Yugoslavia (Lolin, 1957) reported on blood values in 30 pigs, 6 to 8 months of age, which had been vaccinated at various intervals with 5 ml. of crystal violet swine vaccine. No changes in total numbers of WBC's and RBC's were observed; however, an increase in percentage of large lymphocytes and reduction in neutrophils was described. Russian workers (Konopatkin, 1969) describing results of a combined vaccine for use against hog cholera and erysipelas reported that the erysipelas vaccine induced leukocytosis, while leukopenia was present in animals immunized against swine fever with 2.0 ml. of a 1:100 strain "K" line swine fever vaccine. Neither effect was seen in pigs given the combined vaccine. Polish workers (Maciak, 1969) have confirmed the report of Weide and Twiehaus (1959) that vaccination of pigs with lapinized HC vaccines produced leukopenia and eosinopenia.

Dinwiddie (1914) reported that occasionally animals

affected with HC manifested a marked polymorphonuclear leukocytosis due to some inflammatory condition such as pneumonia or pleurisy. The observation was not supported by the work of Lewis and Shope (1929a) who inoculated 2 pigs, which had been infected with HCV 7 days previously, with Bacillus suisepticus. Not only was a leukocytosis absent but the immediate reaction was a more profound leukopenia. Cahill (1929) also investigated this problem by infecting 2 pigs each with Bacillus paratyphosus and the buffalo strain of the hemorrhagic septicemia organism. An acute response characterized by marked leukopenia followed by a leukocytosis in 3 of the 4 pigs was followed by a leukopenia which persisted until death. Kernkamp (1939) described a series of 67 pigs which had developed HC under field conditions. Inflammatory lesions were present in 43 of the animals. The average total WBC count among the 43 pigs was 7,170 while that of the entire 67 was 8,440 cells per cu. mm. of blood. Three of the 24 remaining pigs were heavily infested with ascarids and had TWBC counts of 62,660, 44,440 and 23,300. Sippel (1952) reported on leukocyte counts made on 42 cases diagnosed as swine influenza or pneumonia, 21 cases of swine erysipelas and 31 cases of salmonellosis. None of the total leukocyte counts were below 10,000 WBC per cu. mm. Dunne (1963, 1964a) analyzed the results of leukocyte counts made on 1,671 normal swine 6 to 12 weeks of age. In this group 3.5 percent of the counts were over 40,000 WBC per cu. mm.

and 0.6 percent were under 10,000. The normal range was considered to be 10,000 to 40,000 with the mean at 21,000 WBC per cu. mm. In pigs 6 weeks of age or older, he considered TWBC counts lower than 10,000 as definitely leukopenic and counts of 10,000 to 13,000 as suspicious of HC. During examination of pigs infected for various lengths of time with HC, 2,500 leukocyte counts were made and Dunne (1963) reported more than 53 percent were below 10,000. Analysis of 753 leukocyte counts made on pigs shortly before death from hog cholera indicated that 50 percent of them were below 11,000. Weide and Twiehaus (1959) published a useful summary of early work on the TWBC, TRBC and differential WBC values of swine blood.

The leukopenia produced by HCV is the most widely recognized and utilized for diagnostic purposes of the hematologic alterations observed in HC, however several others have been studied. These include changes in the erythrocyte sedimentation rate (ESR), packed cell volume (PCV), hemoglobin (Hgb), TRBC, differential WBC, bone marrow, and serum proteins.

Erythrocyte sedimentation rate

Although blood changes produced by HCV have been studied since 1910 (King and Wilson, 1910), Lewis and Shope (1929a, 1929b) were the first to comment on the rapid separation of corpuscles and plasma when blood, from febrile pigs affected with HC, was allowed to stand. Kernkamp (1939) reported on

the ESR, measured by the Wintrobe (1935) method, of 36 HCV infected pigs. He found an increase from a normal of 4 mm. per hour to 41.7 mm. per hour on the eighth day postinfection. He concluded that the ESR increased as the disease progressed. Bunce (1954) measured the ESR of 6 normal pigs by the Wintrobe method, and reported an average rate of less than 1 mm. at 1 hour, between 2.25 and 8 mm. at 8 hours and between 5.0 and 11 mm. at 24 hours. Coffin (1945) and Dukes (1955) both reported the ESR of normal swine as between 1 to 14 mm. per hour. Sippel (1952), in a study of methods used for the diagnosis of hog cholera, remarked that although the rate was increased in blood from HC affected animals the test was not reliable or sufficiently definitive. Later Sippel (1958) reported that hog cholera, pneumonia and eperythrozoonosis consistently produced rapid erythrocyte sedimentation rates in swine. Albritton (1952) published ESR values for adult swine of 3 to 8 mm. per hour. Weide and Twiehaus (1959) determined the ESR of normal, ascarid infected and HC vaccinated pigs by the Wintrobe method. Sedimentation rates in the normal animals varied from 0.28 mm. to 2.64 mm. per hour. The rates increased slowly in the ascarid infected pigs. Vaccination with lapinized HC vaccine and challenge of both vaccinated and control animals increased the ESR. The increase after immunization was greater than that following challenge. Schalm (1965) reported ESR's for normal Duroc-Jersey crossbred swine. Animals 3 to 4 months of age had an

average ESR of 2.6 mm. per hour, males 1 year of age had an average rate of 13 mm. per hour, while females of the same age had an average rate of 26 mm. per hour. In each of the aforementioned studies the method used, when it was identified, was that of Wintrobe (1935). This is the only method of measuring the ESR described by most modern texts on veterinary clinical pathology. Benjamin (1961) briefly describes the Westergren and capillary tube techniques but does not list rates for the ESR of swine blood measured by these methods.

Packed cell volume

Few reports describing the effects of HC on the PCV in infected animals have been published. Weide and Twiehaus (1959) reported on PCV values in normal, ascarid infected and HC infected pigs. They obtained PCV values in the range of 20.4 to 32.9 percent for 12 normal animals from 10 to 94 days of age. In both ascarid and HC infected pigs the PCV volume was lower than in the control animals. Sorensen et al. (1961), in describing the hematologic manifestations produced by HC, stated that PCV values closely paralleled Hgb values. Values usually increased from about the fifth to ninth days due to hemoconcentration. They were often reduced, just prior to death, because of terminal hemorrhage. Calhoun and Smith (1964) have cited extensive, pertinent literature on PCV, Hgb, TRBC, TWBC numbers, differential WBC values and serum protein levels of normal swine at various ages.

Hemoglobin concentration

Changes in Hgb values produced by HC infection were among the first effects of the disease observed (King and Wilson, 1910). They reported a decline in Hgb during the course of the disease in proportion to its severity. Lewis et al. (1914) reported the general tendency in cases of HC was a reduction in Hgb and that this was more uniformly present than leukopenia, an opinion not shared by Kernkamp (1939) who reported on Hgb values in 122 pigs infected with HC. He considered a quantitative measurement of Hgb in HC infected swine to be of no particular aid in the clinical diagnosis. Sorensen et al. (1961) stated Hgb values were essentially unchanged during the course of the disease; however, a 1-2 gram percent increase usually occurred around the fifth to ninth day as a result of hemoconcentration due to dehydration. Occasionally in moribund animals a 1 to 2 gram percent decrease due to hemorrhage is detected. Weide et al. (1962), using gnotobiotic and monocontaminated pigs, observed a reduction in Hgb levels during HC.

Total red blood cell numbers

A decrease in the number of erythrocytes in HC infected pigs was reported by most of those who measured this value, from the first study by King and Wilson in 1910 up to the investigations of Kernkamp (1939). Regner (1923) published results which did not agree with those of his contemporaries.

He stated that TRBC numbers did not change in HC infected pigs. Kernkamp (1939) reported that no significant change in TRBC numbers occurred during HCV infection. Weide and Twiehaus (1959) demonstrated that ascarid infected pigs developed a more profound anemia, including erythropenia, than control animals when they were all given a dose of lapinized HC vaccine followed in 16 to 21 days by 4 ml. of a commercial virulent hog cholera virus. Sorensen et al. (1961) published an opinion that was in complete agreement with that of Kernkamp (1939); however, he indicated the TRBC levels made the same minor fluctuations as the Hgb levels.

Differential white blood cell counts

The effect of HCV on various types of WBC's in the peripheral blood has been studied for as long as the leukopenia produced by it. King and Wilson (1910) observed equal reduction in lymphocyte and neutrophil numbers, a smaller reduction in eosinophils and larger increases in large mononuclear cells and transitional forms. Dinwiddie (1914) reported relative increases in large lymphocytes, mononuclears and immature polymorphonuclear neutrophilic leukocytes. A relative increase in lymphocytes of about 75 percent above normal and disappearance of eosinophils from the peripheral blood of HC infected pigs was reported by Regner (1923). In both of their publications, Lewis and Shope (1929a, 1929b) identified the polymorphonuclear neutrophilic leukocyte as the WBC most

severely affected. Using supravital staining, these workers demonstrated that fixed blood smears do not truly represent the actual hematologic picture in HCV infected pigs. Blood containing no neutrophils or eosinophils in fixed preparations frequently contained these cells very badly damaged, extensively vacuolated and apparently very fragile in supravitally stained preparations. Kernkamp (1939) disagreed with previously published work, in that, from the second day of infection there was a larger percentage of neutrophils than lymphocytes in the peripheral blood. Pre-infection hemograms demonstrated an excess of lymphocytes over neutrophils in the same animals. Weide and Twiehaus (1959), studying blood changes in ascarid infected and HC vaccinated pigs, reported that contrary to published results, normal young swine in their study had increasing ratios of neutrophils to lymphocytes from birth to 7 weeks of age. They reported an increase of eosinophils for 12 to 16 days following ascarid infection. Sorensen et al. (1961) described changes in the differential white blood cell picture in hog cholera as quite variable. Mature neutrophils were quickly replaced by immature ones which, in turn, were absent in the terminal stage of the disease. Lymphocyte numbers were quite variable but terminally the relative percentage was usually increased up to 80 or 90 percent to compensate for the terminal neutropenia. Sorensen et al. (1961) summarized his comments that in HC there was both a relative and absolute neutropenia with a relative

lymphocytosis but an absolute lymphopenia and eosinopenia. Weide et al. (1962) pointed out that, with HC infected gnotobiotic and monocontaminated pigs, differential leukocyte counts were not different from pre-inoculation levels.

Bone marrow cells

Varicák (1935) studied the gross appearance of bone marrow of 500 pigs which ranged in age from 9 months to 2 years old. He compared the proportion of fat and red marrow present in different bones at various ages. Noyan (1949) studied the bone marrow cells obtained from the wing of the ilium in 7 swine. Sternal marrow obtained by aspiration with a 16 gauge needle was utilized for the study of bone marrow cells to determine the hematologic manifestations of pteroylglutamic acid deficiency in swine by Cartwright et al. (1948). Cartwright et al. (1949) also used this technique to study the experimental production of a nutritional macrocytic anemia in swine which resembles pernicious anemia in man. Cartwright et al. (1950), using this same technique, described changes in swine bone marrow produced by the administration of 2-6 diaminopurine. Lahey et al. (1952) used the sternal aspiration procedure to study changes in bone marrow from swine which were made deficient in copper. Sternal puncture and aspiration were also used by Köhler (1956) in studying bone marrow cells from large pigs. He preferred the median or lateral tibial tuberosity for studies of bone marrow from

small pigs. Diener[1] carried out preliminary investigations of bone marrow from normal pigs which resulted in a myelogram similar to those obtained by the workers cited above (Calhoun and Smith, 1964). Study of bone marrow cells from pigs infected with HCV was conducted by Sorensen et al. (1961), who described a marked hypoplasia. Nafstad and Nafstad (1965, 1968) have conducted both light and electron microscopic studies on the blood and bone marrow cells from normal and vitamin E deficient pigs. Weber (1969) has presented a thoughtful discussion on the limitations of marrow aspiration or biopsies.

Immature erythrocytes

Regner (1923) was the first to report the observation of increased numbers of nucleated RBC's in the blood of HC infected swine. Lewis and Shope (1929b) reported an increase of nucleated erythrocytes in pigs experimentally infected with hog cholera. They increased from zero on the day of infection to 96 per cu. mm. 2 days later, to a high of 2,124 per cu. mm. 5 days later. While studying the effect of HCV in gnotobiotic swine, Weide et al. (1962) noted that circulating nucleated erythrocytes reached their peak on the seventh day after inoculation but that they did not prevent a fall of Hgb and PCV in the animals.

---

[1]Diener, R. M., Anatomy Dept., Mich. State Univ., East Lansing, Michigan. Tentative studies on the porcine myelogram. Private communication. 1957.

Serum Protein Studies

Moore (1945), during a comparative study of species differences in serum protein patterns, demonstrated differences between electrophoretograms of blood serum from pigs of different ages and from different environments. To achieve optimal separation of protein fractions, buffers of different compounds, pH and ionic strength were required. Serum protein patterns were reported to be so characteristic that identification of the species and, in the case of chickens, the sex, from the pattern was possible. Deutsch and Goodloe (1945) studied electrophoretic patterns of plasma from 20 species of animals obtained under identical conditions. Species variations in mobility, amount and number of protein components were demonstrated. The analytical data were relatively constant for a given species. Immunity to HC was reported to be present in the gamma globulin fraction of the serum proteins by Cameron (1947) and Mathews and Buthala (1960). Electrophoretic changes in serum from swine affected by edema disease consisting of a decrease in the albumin/globulin (A/G) ratio due to a passive loss of albumin and active increase in alpha and gamma globulins or in the gamma globulins alone have been reported by Vesselinovitch (1955). Changes in gamma globulin levels of swine produced by immunization with HCV have been observed by Mathews and Buthala (1955). Greatest changes were apparently due to the increase in gamma globulin after

hyperimmunization. The same workers (Mathews and Buthala, 1956) reported no effect on the electrophoretic pattern of serum proteins, from a pig vaccinated against HC, after the sample had been subjected to 12 freeze-thaw cylces over a 26 week period. Poul (1962) determined the total protein and electrophoretically separated fractions of serum proteins from 12 normal swine. He reported that following hyperimmunization the albumins increased about 50 percent and the total proteins about 20 percent. Neutralizing antibody was reported to be a pseudoglobulin not demonstrable by paper electrophoresis. Knill et al. (1958) and Miller et al. (1961) reported on swine total serum protein levels and electrophoretic patterns of the separated serum fractions collected from normal pigs of various ages. Danilevskii (1960) performed paper electrophoretic separation on serum proteins from 40 healthy young pigs and 47 affected with bronchopneumonia. An increase in gamma and decrease in alpha globulins were observed in the sera of the diseased animals. Weide and King (1962) described changes in serum protein electrophoretograms from young pigs which were passively immune to HC. A sharp decrease in albumin and rise in alpha and gamma globulins were produced by inoculation of virulent HCV. The beta globulins were not affected by infection with the virulent virus. Weide et al. (1962), in another report, suggested that changes in serum protein electrophoretograms appeared to be a more sensitive indicator of the reaction by susceptible pigs to virulent

HCV than leukopenia. He also confirmed their earlier results that HCV infection in susceptible swine resulted in decreased albumin but elevated alpha, beta and gamma globulin values. The discrepancy in the effect on beta globulin values between the 2 reports was not discussed. Turubatović and Panjević (1957) reported on the serum globulin response of pigs vaccinated and revaccinated after various periods for HC. Klimov et al. (1959), while using electrophoretic methods for the purification of HCV, found that the blood from HC infected piglets contained HCV in the alpha, beta and gamma globulins but not in the albumin. Konopatkin (1962) described serum protein changes in vaccinated pigs challenged with virulent HCV. They consisted of increases in the total protein, alpha globulin and beta globulin. The beta globulins decreased after 7 days. The gamma globulin decreased for 12 to 20 days and then increased. Dunne (1964b) considered a hyperproteinemia due to excessive feeding of protein as a significant factor that would increase the susceptibility to HC. Mengeling and Packer (1969), in describing the pathogenesis of chronic HC, published electrophoresis results which are in agreement with those cited previously. Mengeling and Cheville (1968) reported the temporary disappearance of HCV from the blood stream of persistently infected pigs was generally concurrent with increased levels of gamma globulin. As the disease progressed, gamma globulin decreased and virus reappeared in the blood. Terminal decline of gamma globulin was attrib-

uted, by the authors, to progressive depletion of lymphocytic tissues, immunologic exhaustion, or both. Mikhailov et al. (1965) reported on total serum proteins and electrophoretically separated fractions from 100 sows of the Large White and Urzhum breeds. Toward the end of pregnancy albumins increased and globulins decreased. In sows that aborted or produced weak young, gamma globulins were particularly low. Kostyunina and Bychkov (1965) reported a decrease of free amino acids in the blood serum of pigs during the first 6 days after injection of HCV. During this same time total proteins and albumins decreased and beta globulins increased. Alpha and gamma globulins remained unchanged. The great amount of variation and actual contradiction in results of total serum protein levels and their fractions, which were cited here, is explainable to some extent on the basis of the variety of methods, equipment and animals of varying age, breeding and health status, used to derive the data. Unfortunately, precise comparisons are not possible because many of these variables were not adequately defined. Matthaeus and Korn (1969) studied the relationship of immunological changes, TWBC numbers and body temperature changes in pigs affected by HC. First changes in serum proteins occurred in the high molecular weight components. After the body temperature rose and TWBC numbers decreased, an increase in the alpha-2 macroglobulins occurred. The subsequent fall in this fraction was accompanied by a rise in neutrophil leukocytes and formation of

the first 19S (IgM) antibodies. A decline in beta globulins was considered by the authors as due to a fall in transferrin, which coincided with an increase in components of the complement system. Declining levels of gamma globulin were explained as due to the formation of an antigen-antibody complex, by metabolic breakdown of the gamma globulin or its storage in the tissue. Disease specific immunoglobulin was said to be a part of the high molecular weight components of the serum and to be present after the fourth day.

Immunofluorescence Studies

A brief but thorough introduction into the subject of fluorescent antibody (FA) development is presented in the manual by Cherry et al. (1960). Immunofluorescent techniques have been utilized to label specific proteins, including those of viruses and microorganisms, in a variety of tissues. Tissue preparations utilized for FA examination include smears (Goldman, 1954), frozen sections (Coons et al., 1942), and paraffin impregnated sections (Sainte-Marie, 1962). The first FA test (FAT) for the diagnosis of HC was performed using fluorescein isothiocyanate and rhodamine B isothiocyanate labelled swine immunoglobulin on leukocyte smears and cell cultures by Solorzano (1962). Viral antigen was reported to first appear in the nucleus and later in the entire cell. Stair et al. (1963) modified the technique for use on frozen sections and impression smears. Antigen was also reported,

by these workers, to appear in the nucleus as well as cytoplasm of the affected cell. Mengeling et al. (1963a, 1963b) devised an FAT which utilized cells of a pig kidney cell culture line (PK-15). Sirbu et al. (1964) applied the same type of conjugate to lymph node impression smears and detected HCV antigen in 91.1 percent of 32 HC infected pigs. He reported that fluorescence was first apparent in the cytoplasm and later the viral antigen penetrated the nucleus. Cells from several other tissues studied had specific fluorescence in both their cytoplasm and nucleus. Antigen was also described on the surface of erythrocytes. Aiken et al. (1964) reported on the effectiveness of FAT's for the rapid diagnosis of HC, using tissue impressions. Hog cholera virus could be detected in tonsillar tissue 24 to 48 hours earlier than in other tissues. Teebken et al. (1967) reported she was able to differentiate between virulent, attenuated and inactivated HCV by the pattern of fluorescence produced in frozen sections on tonsil tissue. She further observed that the fluorescence due to presence of HCV antigen was restricted to the cell cytoplasm. In the same journal, Aiken et al. (1967) pointed out that the FAT was not specific enough to differentiate between HCV strains. Solorzano et al. (1966), in summarizing results of the FAT using PK-15 cell cultures, reported on 462 cases examined for HCV. The FA procedure was found to be a specific, accurate and rapid method for the routine diagnosis of HC and was considered superior to conventional methods.

Ritchie and Fernelius (1967), while studying HC virions with electron microscopy, described them as between 40 to 50 millimicra in diameter, often asymmetrically shaped and enclosed in a membrane bearing surface projections 12 to 15 millimicra in length. Later they (Ritchie and Fernelius, 1968) confirmed their earlier findings and also described the clumping of HC virions when mixed with fluorescein isothiocyanate tagged swine anti-HCV antibody. Other smaller sized particles were also clumped by the immunofluorescent conjugate. The authors considered the latter particles to be the "soluble antigen" of HCV. This soluble antigen would explain discrepancies between visualization of HC viral antigen by immunofluorescence in a tissue and the inability to grow virus from it. Mayr et al. (1967) and Horzinek et al. (1967) confirmed the work of Ritchie and Fernelius. In addition, Mayr (1967) and his group have confirmed the infectivity of the particles in the 40 to 50 millimicra size range by inoculation of susceptible swine and reproducing classical HC. The FA tissue section technique (FATST) has been used for locating HCV antigen in studies involving host response to HCV (Mengeling and Cheville, 1968), the pathogenesis of chronic HC (Cheville and Mengeling, 1969) and host response in the pathogenesis of chronic HC (Mengeling and Packer, 1969). Tissue culture techniques using the FAT for visualizing HCV antigen have been adapted for studying HCV replication characteristics, (Mengeling and Drake, 1969) and for characterizing in vitro

spread of HCV by cell division (Pirtle, 1969a) and by means of intercellular bridges (Pirtle, 1969b). Sirbu et al. (1968) confirmed HCV can be detected in infected swine lymph nodes which are fresh or after storage at refrigerator temperatures for 2 to 4 days. Kubin and Kölbl (1968), in comparative studies, demonstrated FATST detected only 75 percent of field cases of HC found positive by tissue culture methods. The difference was thought to be due to alteration of viral antigen during shipment. Kubin and Kölbl (1969) later developed a rapid technique for diagnosis of HC in the living animal. Sub-iliac or deep inguinal lymph nodes are surgically extirpated, sectioned at 4 micra and subjected to immunofluorescent study. Infections were detected as early as the second day and results correlated with those of exaltation of Teschen virus (ETV) method. Lin et al. (1969) compared the FA cell culture technique (FACCT) for detecting HCV with the exaltation of Newcastle disease virus (END) method. The FACCT was considered to be as accurate and efficient as the END. Mihajlovic et al. (1969) described results of immunofluorescent investigations which differ from those of most other workers. While tonsil, ileum and spleen are considered preferable for the FAT for HCV, these workers found blood leukocytes, lacrimal gland and lymph node to give best results. No fluorescence was observed in the spleen, lung, liver and kidney. Ressang and DeBoer (1969) developed an indirect FAT for detecting HCV antibody in swine sera. The FA tissue section

and FACCT are the laboratory procedures used by the Animal Health Division, United States Department of Agriculture, as confirmatory tests in the nationwide HC eradication program.

Serum Enzymes

In a multispecies study to determine normal serum glutamic oxalacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) levels, as well as the tissue sources of the 2 transaminases, Cornelius et al. (1959) reported normal serum levels of 31.1 $\pm$ 14.1 units for SGOT and 27.3 $\pm$ 7.8 units for SGPT levels in 1 to 3 year old Duroc pigs. He further noted that SGPT activity was of no value for the diagnosis of hepatic necrosis in swine. Wretlind et al. (1959) determined the levels of glutamic pyruvic transaminase (GPT) in several porcine tissues from normal animals. The concentration of GPT x 10^4 micromoles/gram of wet tissue were: myocardium 0.65; skeletal muscle 0.28; liver 0.18; lung 0.11; kidney 0.10; small intestine and pancreas 0.09 and spleen 0.01. Orstadius et al. (1959) demonstrated an elevation of porcine SGPT in animals affected by liver dystrophy, muscular dystrophy, acute gastroenteritis, femoral neck fracture, enteritis and pneumonia. The elevation was less than four-fold in most cases. Cornelius (1960) reported that administration of carbon tetrachloride with subsequent hepatic necrosis produced marked increases in SGPT activity

in the dog and cat but not in the pig. The same author (Cornelius, 1963) later inferred that hepatic GPT represented the total body activity of the enzyme; however, he was unable to confirm this by producing liver damage and a resulting increase in levels of SGPT. Kolb and Schütz (1961), while studying phosphatase levels in the porcine gastrointestinal tract, found acid phosphatase (AcP) activity predominated over alkaline phosphatase (AP) in the stomach, colon and rectum. In the duodenum, jejunum and cecum the opposite situation occurred. Highest enzyme activity values were found in the ileum, where five times more alkaline than (AcP) was present. Wegger and Westergaard (1963) reported that porcine SGOT and serum alkaline phosphatase (SAP) were age-related but independent of sex. Assays of serum acid phosphatase (SAcP) levels in 157 pigs ranging from 8 to 24 weeks of age were reported by Møller and Westergaard (1964). Average AcP levels were 10.3 King-Armstrong units. Variation in activity levels was much less within litters than between them. Šlesingr (1964) reported results of assays on a variety of serum enzymes in 60 healthy and 32 pigs affected by acute digestive disorders. Pigs with digestive disorders had elevated levels of SGPT, SGOT, serum aldolase and lactic dehydrogenase (LDH) activity. Dougherty et al. (1965) performed assays for SGOT and SGPT on swine infected with virulent Erysipelothrix rhusiopathiae. A significant increase in SGOT levels in all animals with acute swine erysipelas was detected.

However, changes in SGOT in the group affected by the subacute form of the disease and in SGPT in all groups were not significant. Long et al. (1965) determined the AP activity in serum from 41 baby pigs at 1, 2, 7, 14 and/or 21 days of age. Activity generally decreased with age. Storing the serum for 18 hours at 5 C increased the AP activity; however, some of the sera had decreased phosphatase activity after 5 weeks of storage at -20 C. Assays performed by Šlesingr (1965) on sera from 60 pigs weighing between 88 and 132 pounds and affected with pneumonia due to swine influenza, disclosed increased levels of SGOT, serum leucyl aminopeptidase, serum lactic and malic dehydrogenase. During the course of experimental HC, Compagnucci (1966) determined that activity of SGOT, serum aldolase and serum leucine aminopeptidase was increased up to 5 times and serum LDH and AcP were doubled. Witzel et al. (1967) recorded interesting results while studying the effects of inappetence and acute swine erysipelas on glucose and SGOT activity levels in swine. After 3 days of starvation, SGOT levels in 6 normal pigs declined to 82.8 percent of normal. Pigs infected with E. rhusiopathiae had SGOT levels 7 times those of normal animals; however, control pigs on rations restricted in amounts equal to those of the infected animals had SGOT levels which were 71.2 percent of normal. In separate studies, Cassidy and Seberg (1967) in the United States and Compagnucci and Martone (1967) in Italy demonstrated marked elevations in several serum enzymes of

swine infected by strains of HCV of different degrees of virulence. Dressler and Mach (1967) demonstrated an increase of neutrophilic alkaline phosphomonoesterase from the sixteenth to thirtieth day after birth. The alkaline phosphomonoesterase of the eosinophils remained constant. Janda and Pařízek (1967) were unable to demonstrate AP in mucous membranes from the cardiac, fundic and pyloric regions of swine stomachs and were able to demonstrate AcP in those sites only in trace amounts. The specimens were collected by biopsy techniques. Roszkowski (1967) demonstrated a marked increase of AP in adrenal glands collected from swine infected with virulent HCV.

MATERIALS AND METHODS

Hematologic Procedures

Blood collection technique

Blood for all procedures was collected from the orbital sinus (Kuhn et al., 1969) by use of a Micro-Blood collecting tube, 150 mm. long with an outside diameter of 3 mm. and a 1.5 mm. bore.[1] The animal was restrained in a V-shaped trough of appropriate size which was supported on a sturdy table, bench or other stable support. When properly positioned, the animal's head protruded its entire length beyond the end of the trough (Figure 1).

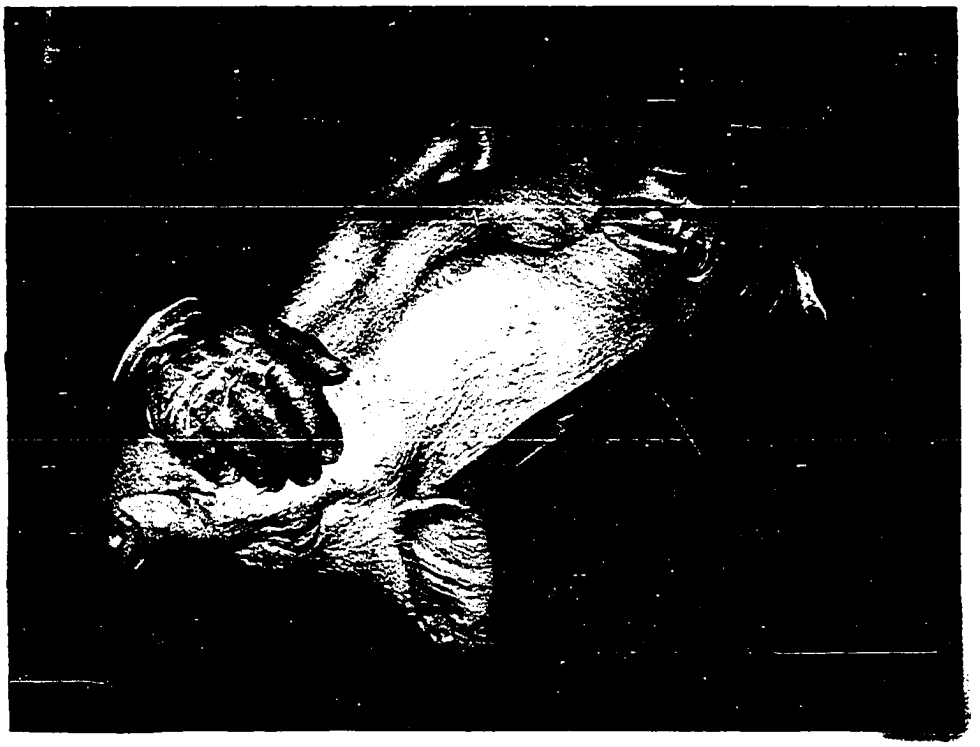
The snout of the pig was grasped with the left hand and the right hand was used to insert the end of the blood collecting tube into the medial canthus (Figure 2). Prior to use, the tapered tip of the tube was etched with a small file around its circumference and broken off approximately 1.0 cm. from the end (Figure 3). After placing the broken end of the tube against the conjunctiva, firm pressure with slight rotation was exerted against the tough conjunctival tissues, which were penetrated. The venous sinus adjacent to the bony orbit was pierced by using rotating pressure on the tube, resulting in a free flow of blood through the tube into a collecting container. Following insertion into the sinus, the tube was

---

[1]Scientific Products Co., Evanston, Illinois.

Figure 1. The pig is restrained on its back in a V-shaped trough which has been placed on the end of a large barrel for support. When properly positioned and restrained, the entire length of the animal's head protrudes beyond the end of the trough

Figure 2. The snout of the pig is grasped with one hand and the other hand is used to insert the end of the blood collecting tube into the medial canthus



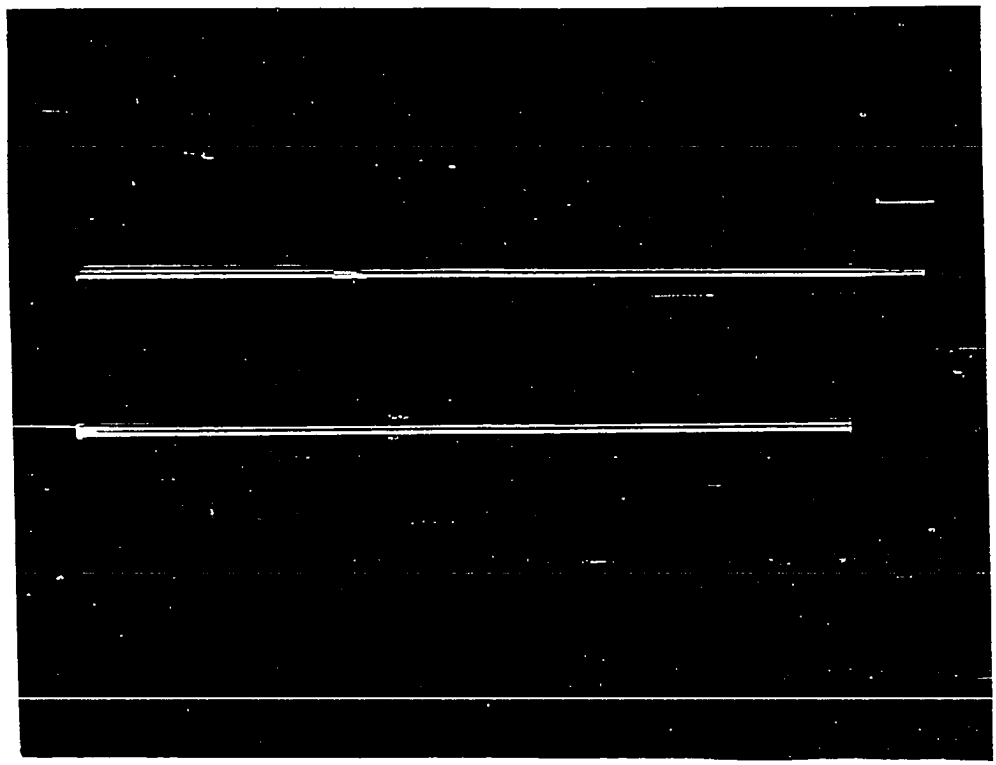


Figure 3. Micro-Blood collecting tube used for orbital sinus bleeding of swine. The tube on top is as purchased and that on the bottom has had its tapered tip removed prior to use

held at an angle which resulted in the free end of the tube being slightly below the level of the eye. Ten ml. samples of blood were usually collected in twenty seconds or less. The tube was withdrawn when the desired amount of blood had been collected and digital pressure was applied with a surgical gauze sponge over the medial conjunctiva to control flow of blood. Restoring the animal to normal position would also produce immediate hemostasis. When daily bleedings were necessary, opposite sinuses were bled on alternate days.

Approximately 5.0 ml. of blood, to be used for determining ESR, PCV, Hgb, TWBC, TRBC, and for preparing reticulocyte smears, was collected in a Vacutainer, a rubber-stoppered evacuated glass container, 100 mm. long and 16 mm. in diameter, which had its internal surface coated with silicone.[1] Prior to use, 2 drops of No-Coag,[2] a concentrated liquid suspension of the anticoagulant, ethylenediamine-tetracetic acid trisodium salt (EDTA or Versene), were added to each tube.

Serum to be assayed for levels of SAP, SAcP, SGPT, SGOT, LDH, and serum proteins was obtained from blood collected in Vacutainers containing no anticoagulant. Within 30 minutes, 10 to 15 Spin-Quik crystals,[2] consisting of chemically inert polystyrene crystals, were added to each 10 ml. of blood and centrifuged at 2000 r.p.m. for 30 minutes. The serum was

---

[1]Becton, Dickinson and Company, Rutherford, New Jersey.

[2]Clini-Tek Specialties, Omni-Tech., Inc., Santa Monica, California.

removed immediately following centrifugation. In most cases, serum enzyme assays and total protein determinations were performed on the day of collection, however in a few instances, where repetitive assays were required, serum was stored overnight at approximately 4 C. All sera from each group of animals was stored at -20 C until completion of that portion of the study. Frozen sera were then thawed and the proteins separated by zone electrophoresis.

To avoid distortion produced by EDTA and other anticoagulants, blood for smears to be used in differential WBC counts was obtained directly from the collecting pipette after the 2 other blood samples were collected.

Erythrocyte sedimentation rate

To determine which of 3 most commonly used methods of measuring the ESR, the modified Westergren method, the Westergren method with undiluted blood or the Wintrobe method, had the highest precision for determining the rate in swine blood, the following study was conducted. Six to 8 ml. of blood was collected from 50 pigs at the National Animal Disease Laboratory (NADL) herd, in EDTA, by the method described above, except that the 3 large castrated male swine were bled by the vena caval method (Carle and Dewhirst, 1942). The ESR was measured on 3 samples of blood by each of the 3 methods, resulting in 9 measurements of the ESR on the blood collected from each animal. All measurements were made within one hour

after the blood was collected.

The order in which tubes of each method were filled was randomized. This was accomplished each day by throwing 3 dice of different colors. Each die was assigned to a particular method and the tubes of the method represented by the die with lowest value were filled first, the tubes represented by the die with the next higher value were filled second, and the tubes represented by the die with the highest amount were filled last. Westergren tubes used for the 2 Westergren methods were randomized by mixing, to reduce any variation between methods due to consistent use of one group of tubes for one particular method. The temperature in the room where the determinations were carried out varied between 22 to 24 C.

Modified Westergren method (Gambino et al., 1965) Two ml. of well-mixed blood were diluted with 0.5 ml. of 0.85 percent (w/v) saline solution. Dilutions were always made just prior to the blood being aspirated into the Westergren tubes (Kimble No. 46735).[1] Immediately after filling, the tubes were inserted into a Westergren rack with 3-footed base and levelling bulb. This rack is designed to maintain the tubes in a vertical attitude (Hellige No. 1000-10S).[2] The rate of fall was observed and recorded one hour after each

---

[1]Kimble Glass Co., Toledo 1, Ohio.

[2]Hellige, Garden City, New York.

tube was filled.

Westergren method with undiluted blood Westergren tubes were filled with undiluted, well-mixed blood and immediately placed in the Westergren rack. The rate of fall was measured by the same timing system as the modified Westergren method.

Wintrobe method (Wintrobe and Landsberg, 1935) The blood sample was mixed carefully and the blood, without air bubbles, drawn carefully into a Wintrobe pipette.[1] The pipette was then inserted to the bottom of the Wintrobe tube.[1] The blood was expelled gradually, keeping the tip of the pipette below the surface of the blood until the 0 mark was reached. The tube was then placed in a Wintrobe vertical rack[1] and read and recorded as described under the modified Westergren method.

Modified Westergren versus dilute Wintrobe method Although the modified Westergren method was the most consistent method of measurement of the 3 methods compared with blood from healthy pigs, it was necessary to demonstrate if it was sufficiently sensitive to measure ESR of blood from HC infected swine, therefore the following study was conducted.

The blood sample was diluted by mixing 2 ml. of well-

---

[1]Scientific Products Division, American Hospital Supply Corp., Evanston, Illinois.

mixed blood with 0.5 ml. of 0.85 percent (w/v) saline solution. Dilutions were made just prior to filling the tubes. Two tubes were filled as described under the respective methods in the previous comparative study. The distance of erythrocyte sedimentation was observed and recorded one hour after the tubes were filled.

Packed cell volume

The PCV, or cell pack, was determined by use of the micro-hematocrit method. A plain capillary tube,[1] 75 mm. long and 1.3-1.5 mm. in diameter was filled with versenated blood. The vacant end of the tube was closed with Seal-Ease,[2] a special clay sealant compound. Tubes were then placed in the slots, with open ends toward the hub and sealed ends as close as possible to the rubber cushioned inner rim, of the hub of an International I.E.C. Capillary Centrifuge, Model MB.[3] The capillary tubes were then centrifuged at a speed of 11-15,000 r.p.m. for 5 minutes. The tubes were then removed and the PCV was read directly on an Adams Micro-hematocrit Reader No. A-2970[2] by the following procedure:

1. A capillary tube was placed in one of the grooves in the moveable plastic holder and the bottom of the packed red cells aligned with the base line of the holder.

---

[1]Aloe Scientific, Division of Brunswick Corp., St. Louis, Missouri.

[2]Clay Adams, Inc., New York 10, New York.

[3]International Equipment Company, Boston, Massachusetts.

2. The plastic holder was then moved to the right or left until the top of the plasma in the capillary tube coincided with the top line (100 percent) on the top of the white triangle.

3. By moving the knob on the end of the reader bar, the line on the reader bar was placed at the junction of the packed cells and plasma.

4. The percentage of packed red cells was then read from the Micro-hematocrit reader window.

Hemoglobin concentration

Hemoglobin levels in all blood samples were determined by the cyanmethemoglobin method. The assay was conducted with reagents in ready to use form. A standard curve that was used to determine grams of Hgb per 100 ml. of blood was constructed in the following manner:

1. Five ml. of Acuglobin Hemoglobin Standard[1] was pipetted into a clean dry cuvette. (Acuglobin Hemoglobin Standard is a cyanmethemoglobin solution which is kept in constant equilibrium with its gas phase to assure maximum accuracy by being hermetically sealed in a hard glass ampule.) The Acuglobin was allowed to reach room temperature before use. After photometric readings were recorded, the standard was discarded. Each standard has a specific Hgb concentration which is usually around 60 mg. cyanmethemoglobin per 100 ml.

---

[1]Ortho Diagnostics, Raritan, New Jersey.

2. Another 2.5 ml. of Acuglobin was pipetted into a second clean dry cuvette. The pipette was then rinsed several times with Aculute Diluent.[1] The diluent was prepared by dissolving one Aculute Diluent Pellet in 250 ml. of distilled water. The active ingredients in the diluent pellets are sodium bicarbonate, potassium ferricyanide and potassium cyanide. Using the same pipette, exactly the same volume (2.5 ml.) of Aculute Diluent was added to the second cuvette. This 1:1 dilution of cyanmethemoglobin then contained 30 mg. of cyanmethemoglobin per 100 ml.

3. A blank was prepared by adding 5.0 ml. of Aculute Diluent to a third cuvette.

4. All photometric readings were made in a Hitachi Perkin-Elmer, Model III UV-VIS Spectrophotometer.[2] The blank was placed in the instrument and the wave length scale set at 540 mμ and transmission at 100 percent.

5. The cuvette containing the undiluted Acuglobin was then placed in the instrument and percent transmission recorded.

6. The cuvette filled with diluted Acuglobin was then measured in the instrument and percent transmission recorded.

7. Percent transmission was then converted to optical density by use of appropriate conversion tables.

---

[1]Ortho Diagnostics, Raritan, New Jersey.

[2]Hitachi Limited, Tokyo, Japan.

8. A standard curve was prepared on linear coordinate graph paper. Hemoglobin concentrations, which ranged from 0 to 20 grams per 100 ml., were placed on the horizontal axis. The optical densities, ranging from 0.00 to 0.500 were placed on the vertical axis. The optical density and equivalent gram percent hemoglobin values for the undiluted and diluted Acuglobin Hemoglobin Standard were used to plot construction points.

Hemoglobin concentration in blood samples from experimental animals was determined as follows:

1. Exactly 20 microliters of versenated blood was diluted in 5.0 ml. of Aculute Diluent (1:251 dilution). The reaction was allowed to proceed for 10 minutes before the percent transmission was determined photometrically. The optical density was determined from conversion tables.

2. Using the standard curve graph, a straight line from the correct optical density value on the left vertical axis was drawn parallel to the horizontal axis until it intersected the standard curve. A line from the intersection parallel to the vertical axis was drawn down to the horizontal axis where the correct value, of Hgb in grams per 100 ml. of whole blood, was read.

A new standard curve was constructed when each new batch of Aculute Diluent was prepared.

Total white blood cell counts

All total white and red blood cell counts were made using a Model B Research Counter with a 100 micra aperture.[1] Aperture current and threshold values which accurately enumerated swine WBC's in a blood sample had been previously calibrated by the method of Wisecup and Crouch (1963). The following procedure was followed:

Machine settings:

Amplification	2	
Aperture current	½	
Threshold	Lower	8
	Upper	102

Machine operation:

The machine was switched on at least 10 minutes before use. Counts made prior to this time were frequently inconsistent.

Between periods of use the electrodes and aperture tube were left immersed in Isoterge,[1] a blended detergent in a balanced isotonic solution with a neutral pH and minimal hemolytic properties.

Just prior to use the entire system of the Coulter Counter was flushed with Isoton[1] an appropriately balanced, isotonic solution of electrolytes which contains 0.1 percent sodium azide as an antimicrobial agent.

Three counts were made to determine the background counts of particles present in the Isoton diluent. It was necessary to enclose the entire sample holding stage area with heavy aluminum foil in order to get background counts of 50 or less which was considered satisfactory for leukocyte counting.

Blood samples to be counted were diluted in Isoton in the following manner.

---

[1]Coulter Electronics, Inc., Hialeah, Florida.

The sample blood was mixed thoroughly and 0.2 ml. were removed in a pipette and transferred to a 100 ml. volumetric flask partially filled with Isoton. After addition of the blood the flask was filled to volume with Isoton.

Thirty drops of Zap-Isoton[1] were promptly added to the volumetric flask. The contents were mixed thoroughly.

Total WBC counts were performed immediately using a 50 ml. beaker to hold the prepared sample for aspiration through the measuring aperture.

White blood cell counting procedure:

The counting chamber of the Coulter Counter was flushed thoroughly with Isoton.

The mercury column was then allowed to drop to the precount level.

Reset lever was activated and the zero level checked on all counting dials on control panel.

Each sample was counted 3 times and the mean value determined. If the value was over 15,000 WBC/cu. mm. it was corrected for coincidence from a chart supplied by the manufacturer. This was necessary due to the machine's operating characteristics, which result in its reading 2 or more white or RBC's as 1 when they pass through the counting aperture concurrently. The total number of WBC's in each sample was corrected for the number of nucleated erythrocytes observed during the differential count of a smear made from the same blood sample.

The system was flushed with Isoton again, after which the machine was ready to count the next sample.

Total red blood cell counts

The same Coulter Counter was also used to perform TRBC counts. The machine aperture current and threshold values

---

[1]Coulter Diagnostics, Inc., Hialeah, Florida.

for RBC's were also calibrated by the method of Wisecup and Crouch (1963). The following values were used:

Machine settings:

Amplification	1	
Aperture current	0.354	
Thresholds	Lower	10
	Upper	102

Machine operation:

The machine is prepared in the same manner for making erythrocyte counts as for leukocyte counts.

The blood sample was diluted as follows for making TRBC counts.

After thoroughly mixing the blood sample, 0.02 ml. of blood is transferred via a Hgb pipette into an 18 x 140 mm. test tube filled with 10 ml. of Isoton.

One ml. of this mixture was then transferred by a volumetric pipette into a 100 ml. volumetric flask partially filled with Isoton. After addition of the blood and Isoton, the volumetric flask was filled to volume with Isoton and mixed thoroughly.

Samples were transferred to 50 ml. beakers for counting, as this facilitated immersion of the aperture tube into blood and Isoton mixture.

The counting procedure was the same as for making leukocyte counts except the final value was multiplied by 100 and corrected from the coincidence chart supplied by the machine manufacturer.

Differential leukocyte counts

Preparation, examination and interpretation of blood smears from the experimental animals were conducted using procedures which are described in the following paragraphs:

Preparation of slides:

Blood for making smears to be used in differential WBC counts was taken directly from the eye bleeding pipette. A small drop was placed on a glass microscope slide and spread with the smooth, short edge of another slide according to the method of Schalm (1965). Slides were allowed to completely air dry, and were stained within 3 hours after the blood was collected.

Wright's Blood Stain[1] was flooded over the smear and allowed to stand for 5 minutes.

A buffer[2] prepared specifically for Wright's stain was then added to the stain on the slide. Mixing of the stain and buffer was accomplished by gently blowing on the slide until a characteristic metallic sheen appeared on the surface. This mixture was left on the smear for 8 minutes.

The stain and buffer were then poured off of the smear and it was thoroughly rinsed with double distilled water.

The bottom of the slide was then wiped clean of stain and the slide leaned lengthwise against a test tube rack and allowed to dry.

Counting and interpretation of slides:

Differential counting was performed by the cross sectional method of MacGregor, Richards and Loh (1940).

Total numbers of cells counted was determined on the basis of the total number of leukocytes in the blood sample. One hundred cells were differentiated when the total count was 10,000 cells or less and 200 were counted when the TWBC numbers in the sample exceeded 10,000.

The cells were differentiated on the morphologic and staining characteristics described by Schalm (1965).

---

[1]Wright's Blood Stain, Item No. 740, Hartmann-Leddon Co., Philadelphia, Pa.

[2]Buffer Salt Mixture pH 6.8 for Buffering Blood Stain Reaction, Item No. 4022, Hartmann-Leddon Co., Philadelphia, Pa.

Bone marrow evaluation

Aspiration of bone marrow from the sternum of the pigs, preparation of bone marrow smears and the method of examination are described in the following section.

Specimen collection:

Bone marrow samples were collected immediately after animals were bled from the orbital venous sinus for hematologic studies. The following procedure was used for collection of all bone marrow samples:

Animals to be sampled were placed on their backs in a V-shaped trough. The rear legs and right front leg were held by one assistant while a second assistant held the left front leg and steadied the position of the head. (See Figure 1.)

The skin over the anterior one-third of the sternum was wiped clean with sterile gauze, liberally sponged with 50 percent ethanol and wiped dry with sterile surgical sponges. Strong Iodine Tincture N.F. was then painted over the surgical site and allowed to dry. At this point the operator changed to sterile surgical gloves and, from this point on in the procedure, aseptic operative technique was practiced.

A University of Illinois Biopsy Needle,[1] pediatric size 18 ga. x 1 inch, was adjusted until the needle shaft protruded approximately 2 cm. from the shield (Figure 4). Sterile technique was also maintained during this manipulation.

The sternum was palpated until the shaft of the first or second sternebra was located and marked with the left index finger (Figure 5).

While steadying the sternum with the left hand, the shaft of the biopsy needle was aligned with the right hand at a 45 degree angle with the side of the sternebra (Figure 6).

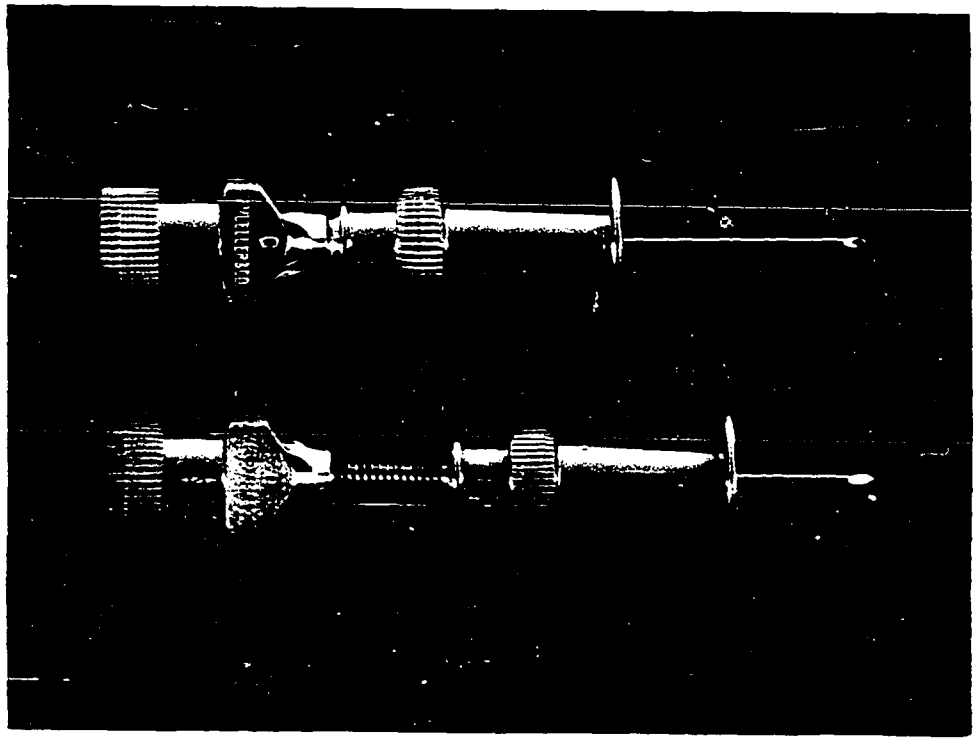
The needle point with its acute angle presented ventrically was firmly pushed into and through the skin

---

[1]V. Mueller and Co., 1929 Second St., S.W., Rochester, Minnesota. Catalog No. SU-21001.

Figure 4. University of Illinois biopsy needle before (top) and after (bottom) adjustment for use in aspirating bone marrow from the sternum of young pigs

Figure 5. While palpating the sternum with the left hand to locate the first or second sternebrae, initial penetration of the skin and subcutaneous tissue is accomplished with the biopsy needle in the right hand



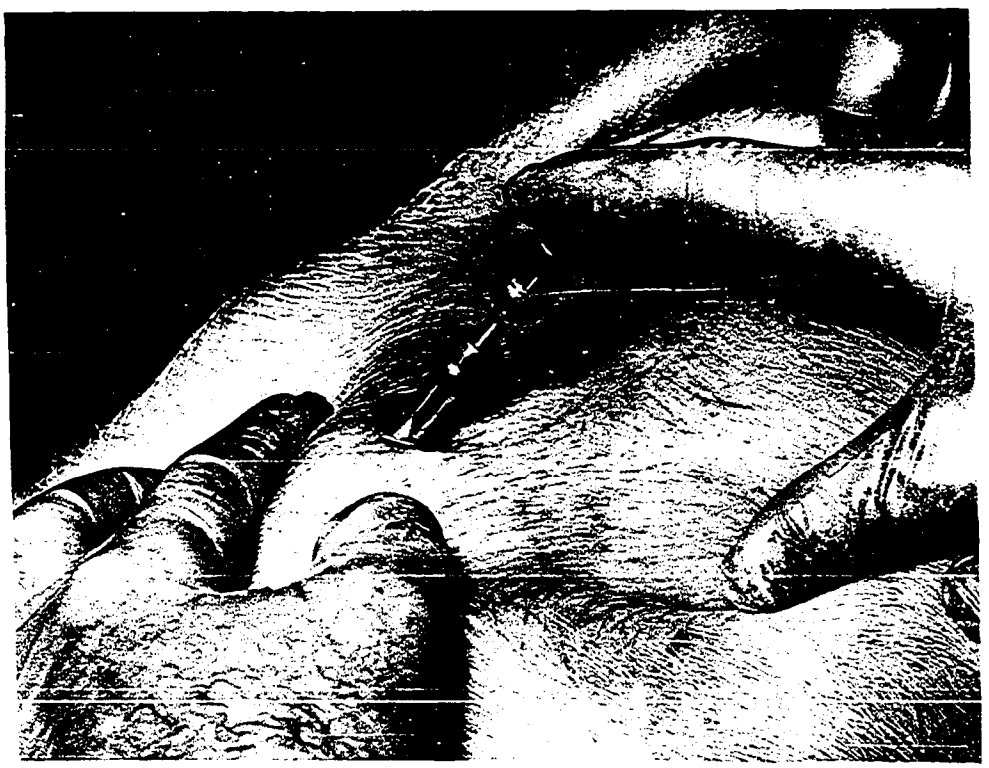


Figure 6. The biopsy needle has been inserted at a 45 degree angle with the side of the sternebrae while the animal is being restrained on its back in a V-shaped trough

subcutis, muscle and periosteum. When bone was felt against the needle point the biopsy needle was rotated in one-half to three-fourths of a turn until the needle was felt to suddenly move into tissue offering little resistance.

Immediately the stylus in the shaft of the biopsy needle was unscrewed and removed.

A clean, dry 20 ml. disposable plastic syringe[1] was quickly attached to the biopsy needle. A firm and constant tension was maintained on the plunger until several drops of bone marrow were observed to ooze into the barrel of the syringe (Figure 7). Bone marrow was obtained on the first try in approximately 80 percent of the collection attempts using this technique.

Smear preparation:

The marrow sample was immediately expelled onto a glass plate 20 x 20 cm. The most likely looking small pieces of bone marrow were aspirated into a Pasteur pipette and deposited on clean glass microscope slides.

The small deposit of bone marrow was then spread over the slides, air dried, and stained as previously described for peripheral blood smears used for differential WBC counts. Marrow to be stained by the FATST was spread and immediately air dried. The FATST is described under preparation of tissue sections.

Criteria used for identifying and classifying bone marrow cells are those of the Committee for Clarification of the Nomenclature of Cells and Disease of the Blood Forming Organs (1949).

Many of the samples collected during leukopenic stages of the disease contained very small numbers of granulocytic and erythrocytic precursor cells. To assure that a representative sample of these cells were counted, the following guidelines were adhered to:

A minimum of 300 cells were evaluated from all samples.

---

[1]Pharmaseal Laboratories, Glendale, California, Catalog No. 7420.

Figure 7. A 20 ml. plastic syringe has been attached to the biopsy needle. Constant tension is maintained until several drops of bone marrow are aspirated into the syringe

No less than 200 of these cells were required to be nucleated cells of the erythrocytic and/or granulocytic series.

Cells were catalogued as indicated in the charts in the results section.

Reticulocyte counts

Reticulocytes were enumerated in blood smears prepared by the following procedure:

Reticulocyte stain:

Brilliant cresyl blue[1] was utilized at 1 percent concentration in 0.85 percent saline with 0.4 gm. of sodium citrate added.

An equal number of drops of this brilliant cresyl blue solution and blood were placed in 15 x 140 mm. pyrex test tubes and mixed thoroughly.

Cells were allowed to stain for a total of 20 minutes.

A small drop of the mixture was placed on one end of a glass slide with a wooden applicator stick.

The drop was spread with the edge of another slide to produce a thin film which was dried leaning against a test tube rack.

Wright's stain was used as a counterstain using the same staining procedure employed for differential WBC count.

Counting reticulocytes:

The percentage of reticulocytes in approximately 500 erythrocytes was determined by examination of the smear, using an oil immersion objective.

The number of erythrocytes was estimated by locating an area in the smear, near the edge, where erythrocytes were uniformly distributed. The number of red

---

[1]Brilliant cresyl blue C.I. No. 51010. National Biological Stains Dept., National Aniline Div., Allied Chemical Corporation, Harristown, N.J.

blood cells in 3 microscopic fields were counted and an average obtained. If differences between the number of cells in these 3 fields was greater than 20 percent of the total in the first field, two additional fields were counted and an average of 5 fields was obtained.

The resulting average number of cells per field, divided into 500, was the number of microscopic fields counted on that slide.

They are reported in percentage, or reticulocytes per 100 cells.

Howell-Jolly bodies

The percentage of Howell-Jolly bodies was determined on the blood smear used for enumerating reticulocytes. They were counted concurrently with reticulocytes and reported in the same units.

Serum Protein Studies

Total serum protein determinations

Measurement of total serum proteins was by the biuret method. The biuret reagent was prepared by the method of Gornall *et al.* (1949). A standard protein curve was established using a protein standard solution[1] consisting of crystalline bovine albumin with an assayed potency of 9.8 milligrams of protein nitrogen per milliliter. This is the equivalent of 61.25 milligrams of protein per milliliter due to the standard value of nitrogen in protein, e.g. (9.8 mg./ml. of N x 6.25=61.25 mg. of protein/ml.).

---

[1]Armour Pharmaceutical Co., Chicago, Illinois.

Calibration of standard curve A curve describing the total serum protein concentration with percent light transmission measured in a spectrophotometer was calibrated by the procedure described below.

> A calibration curve was prepared by pipetting 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1.0 ml. of standard protein solution into 15 x 140 mm. test tubes. Then in the same order 9.9, 9.8, 9.7, 9.6, 9.5, 9.4, 9.3, 9.2, 9.1, and 9.0 ml. of 0.9 percent saline were added to the same tubes.
>
> Into another group of 10 test tubes 0.5 ml. of each of the serum-saline mixtures were transferred. This was diluted with 9.5 ml. of 0.9 percent saline and the dilutions mixed thoroughly and 2.0 ml. transferred to another group of 10 test tubes.
>
> A blank was prepared by placing 2 ml. of 0.9 percent saline in a test tube of the same dimensions.
>
> Eight ml. of biuret reagent were added to the 11 test tubes and mixed thoroughly.
>
> The tubes were left to stand for 30 minutes and then read in percent light transmission (%T) at a wavelength of 540 mμ in a Hitachi Perkin-Elmer, Model III UV-VIS Spectrophotometer.
>
> Values were then converted to optical density values from a standard conversion chart.
>
> Density values were plotted against protein concentrations of the original dilutions. When calculated using the dilutions outlined, these gave concentrations of 1.2, 2.4, 3.7, 4.9, 6.1, 7.3, 8.6, 9.8, 11.0 and 12.2 gms. per 100 mls. of protein.

Standard biuret method The biuret method of determining total serum proteins used for both standard curve calibration and determining protein concentration in the blood from the experimental animals was conducted by the following procedure.

Dilute 0.5 ml. of the unknown serum with 9.5 ml. of 0.9 percent saline and mix thoroughly.

Place 2.0 ml. of diluted serum in a test tube. In another tube place 2.0 ml. of 0.9 percent saline to be used as a blank.

Add 8.0 ml. of biuret reagent to both tubes and mix.

Let the tubes stand for 30 minutes. Read the percent transmission of the sample against the blank with spectrophotometer set at a wavelength of 540 mμ.

Results are converted from percent transmission to optical density and the final results read directly from the standard curve.

Electrophoretic studies

All electrophoretic examinations of swine sera were conducted by the method of Kaplan and Savory (1965) and Grunbaum et al. (1963) with a Microzone cellulose acetate electrophoresis system[1] which accommodated eight serum samples on a single membrane. This used about 0.2 μl of serum per sample and required 20 minutes of electrophoresis to separate the protein fractions. The strips were stained, cleared to transparency and scanned and recorded by a Microzone Densitometer, Model R-110.[1]

Fluorescent Antibody Procedures

A direct FA staining technique (Stair et al., 1963, 1964) was used for detection of HCV antigen in tonsil biopsy tissue, bone marrow smears, and sections of tonsil, prepectoral lymph

---

[1]Spinco Division, Beckman Instruments, Inc., Palo Alto, California.

node, spleen and ileum collected at necropsy.

The FA conjugate was obtained from Dr. William C. Stewart of the Virology Section, Diagnostic Services, NADL, Ames, Iowa. It was prepared according to the method of Mengeling et al. (1963a, 1963b).

Tissue sections

All tissues examined by FA procedures were frozen in an International Harris Cryostat, Model CTD.[1] The tissues were attached to cryostat microtome buttons for sectioning with Tissue Tek O. C. T. Compound No. 4583,[2] an embedding medium for frozen tissue specimens. Frozen sections were cut at 8 microns, mounted on cytology micro slides completely frosted on one side,[3] and fixed immediately in acetone[4] at room temperature for at least 10 minutes.

Bone marrow smears

After being thoroughly air dried, bone marrow smears were stored from 2 to 6 hours in a refrigerator at 5 C and then fixed in acetone at room temperature for at least 10 minutes. From this point in the FATST, the same procedure was used for both tissue sections and bone marrow smears.

---

[1]International Equipment Company, Needham Heights, Massachusetts.

[2]Ames Company, Elkhart, Indiana.

[3]Clay Adams, Inc., New York 10, New York.

[4]Mallinckrodt Chemical Works, St. Louis, Missouri.

Solutions

Reagents required in the fluorescent antibody tissue section technique required careful preparation to obtain optimal results. The following procedures produced the best results under the conditions of this study.

Carbonate buffer:

Solution A -	Na_2CO_3	53.0 gm.
	Distilled water q. s.	1000.0 ml
Solution B -	$NaH\ CO_3$	42.0 gm.
	Distilled water q. s.	1000.0 ml.

Mix 800 ml. of solution A and 215 ml. of solution B to produce a buffer with a pH of approximately 9.0. The pH was checked on an IL Delta-matic pH/mV electrometer.[1]

Carbonate buffered saline:

Carbonate-bicarbonate buffer	1000.0 ml.
Sodium chloride	8.5 gm.

The solution was kept tightly stoppered when not in use. It was easier to maintain the desirable pH when small amounts of carbonate buffered saline were prepared frequently, rather than large amounts at one time.

Buffered glycerin:

Glycerin, U.S.P.[2]	90.0 ml.
Carbonate buffered saline	10.0 ml.

This produced a buffered glycerin with a pH of 9.0

---

[1]Instrumentation Laboratory, Inc., Boston, Massachusetts.

[2]Mallinckrodt Chemical Works, St. Louis, Missouri.

Staining procedure

The immunofluorescent demonstration of HCV antigen in the tissues of the infected animals was accomplished by the following technique.

> Acetone-fixed, frozen tissue sections and bone marrow smears were flooded with anti-HCV, FA conjugate and incubated in a moist chamber at 37 C for 30 minutes. This moist chamber consisted of a covered petri dish containing moist cotton and two pieces of applicator stick on its bottom to support the microscope slides. These were placed in a Model IMP-732 Incubator.[1]
>
> Slides were actively washed for 10 minutes with 3 changes of carbonate buffered saline or were washed for 10 minutes in a mechanical washer. Basically, this consisted of a spring loaded rack which, when inverted over a 400 ml. Pyrex beaker,[2] held up to 20 slides in the buffer solution. Washing was accomplished by placing a Teflon covered magnetic bar in the buffer in the beaker which was placed on a Magnestir,[3] magnetic stirrer.
>
> Slides were then rinsed in distilled water and each slide cover-slipped with glycerin buffered to a pH of 9.0.

At the same time each group of tissue sections or bone marrow smears were stained by the FAT, a section of HCV infected tissue, usually tonsil, was also stained. This served as a control on the procedure, to be certain that viral antigen was detected, if present, in the samples checked.

---

[1]Chicago Surgical and Electrical Company, Chicago, Illinois.

[2]Aloe Scientific, Division of Brunswick Corp., St. Louis, Missouri.

[3]Scientific Products, Inc., Evanston, Illinois.

Inhibition

The two step inhibition method described by Cherry et al. (1960) was used to confirm specificity of the FA reaction on both tissue sections and bone marrows. In addition to the slide from each animal stained by the regular method, 2 additional smears or tissue sections were prepared as follows:

> One slide was flooded with normal porcine serum and the other with unlabelled HC antiserum. Both slides were incubated at room temperature in a moist environment for 40 minutes.
>
> The sera were removed from both slides by washing in carbonate buffered saline, pH 9.0.
>
> Both slides were flooded with FA conjugate and incubated in a moist environment at 37 C for 15 minutes.
>
> A vigorous washing in carbonate buffered saline for 10 minutes followed for both slides.
>
> All slides were rinsed in distilled water.
>
> Each slide was cover-slipped with buffered glycerin and examined with the aid of an ultraviolet microscope.

An absence or marked reduction of fluorescence was observed on sections treated with HC antiserum. Fluorescence due to HC viral antigen was present on the section treated with normal swine serum. A reduction in intensity of fluorescence in sections containing viral antigen treated by normal swine serum was noted, which was probably due to dilution of the conjugate by serum or the additional washing required in the inhibition procedure.

Microscope

A Leitz Orthoplan microscope[1] with high pressure mercury burner HBO 200, type L, II and heat sink was used. This included 2 heat absorbing filters, KG 1 and BG 38, a UGI ultraviolet excitation filter and a BG 12 blue light excitation filter.

Tonsil Biopsy Procedure

The animal was restrained by being elevated by the front legs with its abdomen turned toward the operator. Its shoulders and hips were clamped by the legs of the assistant holding the animal's front legs. A second assistant inserted a swine mouth speculum[2] and opened the mouth by exerting gentle but firm downward pressure on the speculum handle. Concurrently the second assistant, standing slightly to the animal's right and operator's left, held a flashlight to illuminate the tonsillar area. Swine tonsils are located on either side of a median furrow on the oral surface of the soft palate, and appear as slightly raised areas which are distinctly paler pink than surrounding pharyngeal tissue. Using Welch-Allyn rectal biopsy forceps[3] (Figure 8), which are 14 inches long

---

[1]E. Leitz, Inc., New York, New York.

[2]Haver-Lockhart, Shawnee, Kansas.

[3]Physicians and Hospital Supply Co., Inc., Minneapolis 3, Minnesota.

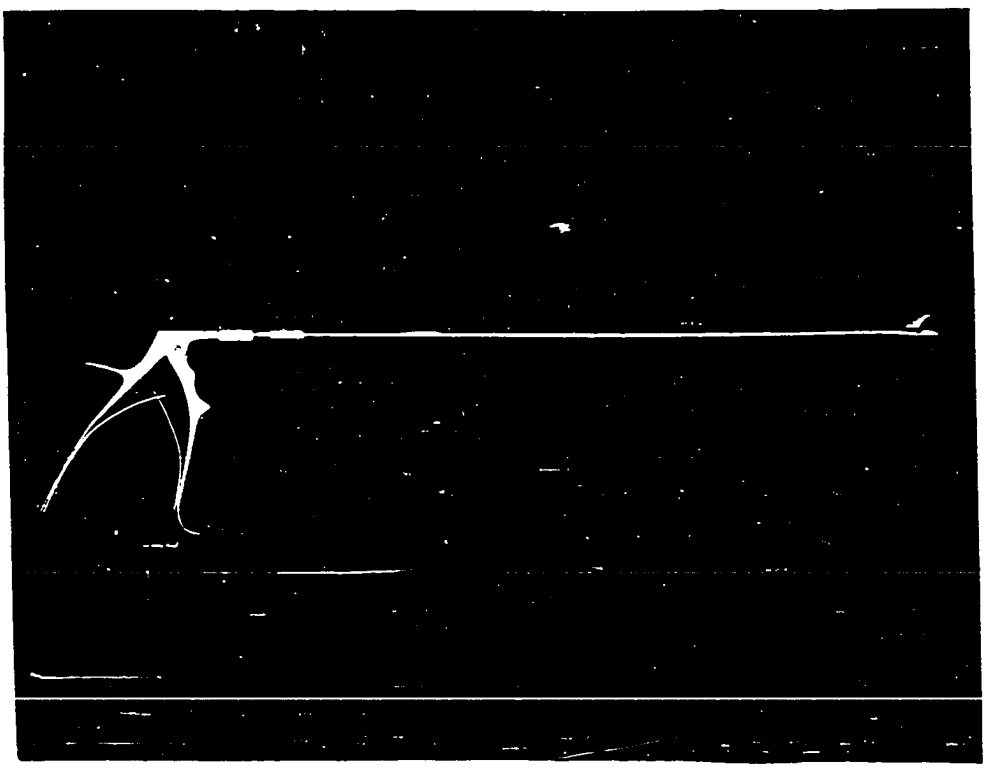


Figure 8. Welch-Allyn rectal biopsy forceps used for collection of tonsil tissue

with straight jaws, the operator opened the jaws of the instrument and firmly forced them into the tonsil tissue and then quickly closed them. Biopsy tissues were placed in small plastic bags and immediately stored at 5 C until they were sectioned and stained, usually the day of the biopsy.

Necropsy Procedures

All animals not dying within the experimental period, or which were moribund, were euthanatized by vena caval injection of 5 ml. of a 10 percent solution of succinylcholine chloride.[1] Animals were exsanguinated while under the influence of the drug and a complete necropsy performed immediately. Slices of tonsil, prepectoral lymph node, spleen, and ileum approximately 0.5 cm. thick were collected immediately. These were placed in small plastic bags in a refrigerator at 5 C until sectioned and stained. A careful examination of all organs and tissues was then conducted. Representative sections of all major organs and tissues were collected. In addition, appropriate samples of all lesions noted were removed for later histologic study.

---

[1]Sigma Chemical Company, St. Louis, Missouri.

Histologic Procedures

Tissues collected at necropsy, other than those examined by the immunofluorescent technique, were fixed in 10 percent buffered formalin for a minimum of 96 hours. Sections of bone were decalcified using Decal[1] for 12 hours, then washed in running tap water for 6 hours. All tissues were dehydrated in graded ethanol solutions, cleared in xylene, and embedded in paraffin. Sections were cut at 6 μ and mounted on glass slides with Permount[2] mounting medium. All sections were stained with Harris's hematoxylin and eosin Y. Selected sections were stained by the periodic acid-Schiff reaction and MacCallum-Goodpasture's bacterial stain. These procedures were carried out as described in the Armed Forces Institute of Pathology's Manual of Histologic and Special Staining Techniques (1968).

Serum Enzyme Assay Procedures

All serum enzyme assays were performed on the day of collection, with the only exceptions being, that in several cases, repeat assays were made after the serum had been stored overnight at 4 C. Recently, the International Union of Biochemistry (1965) recommended the systematic naming of enzymes

---

[1]Scientific Products Division, Hospital Supply Corp., Evanston, Illinois.

[2]Fisher Scientific Company, Fair Lawn, New Jersey.

and coenzymes. At the time this study was in progress, the new nomenclature had not gained wide acceptance, therefore the more familiar designations will be used. The table below is provided to facilitate identifying the same enzyme by its several names.

Table 1. Enzyme nomenclature

	Old name	New name	New complete name
1.	Alkaline phosphatase	Alkaline phosphatase	Orthophosphoric monoester phosphohydrolase
2.	Acid phosphatase	Acid phosphatase	Orthophosphoric monoester phosphohydrolase
3.	Glutamic pyruvic transaminase	Alanine aminotransferase	L-alanine: 2 oxoglutarate aminotransferase
4.	Glutamic oxaloacetic transaminase	Aspartate aminotransferase	D-aspartate: 2 oxoglutarate aminotransferase
5.	Lactic dehydrogenase	Lactate dehydrogenase	L-lactate: NAD oxidoreductase

Serum alkaline phosphatase

Determination of serum levels of this enzyme were conducted with commercially available reagent kits and according to Sigma Technical Bulletin No. 104,[1] which is based on the work of Bessey et al. (1946), Andersch and Szczypinski (1947), and Fujita (1939). The principle of this test is based upon the hydrolysis at a pH of 10.5, of p-nitrophenyl phosphate. When this compound, which is colorless in either acid or alkaline solution, is hydrolized by the phosphatase enzyme, the phosphate group is split off the parent compound and the yellow salt of p-nitrophenyl is liberated. The salt is yellow only in alkaline solution, which results when the reaction is terminated by addition of sodium hydroxide. The substrate itself serves as the indicator of the amount of hydrolysis occurring during a unit of time and consequently the concentration of phosphatase present. Results were reported in Sigma units. One Sigma unit of phosphatase will liberate 1 micromole of p-nitrophenol per hour, under conditions specified in the assay procedure.

Serum acid phosphatase

Both acid and AP's hydrolyze the same substrates. It is the range of pH at which the two enzymes are most active that constitutes their major difference. Consequently, SAcP

---

[1]Sigma Chemical Company, St. Louis, Missouri.

was determined by essentially the same materials and method with a few minor differences outlined in Sigma Technical Bulletin No. 104. The same substrate solution was used in both assays, however, the SAcP determination required an acid buffer in place of the alkaline buffer used for the SAP determination. The reaction was terminated by addition of a stronger concentration of sodium hydroxide than with SAP assay and a separate serum blank tube is required. The latter was not required in SAP determination, where the serum assay and reagent blank tube are acidified to provide correction factors for the serum. Results were reported in the same Sigma units which SAP are reported.

Serum glutamic pyruvic transaminase

Glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT), the two most important transaminases found in blood, both catalyze transfer of an amino group from an alpha-amino acid to an alpha-keto acid forming a new amino acid and a new keto acid. These enzymes were both assayed by the colorimetric method of Reitman and Frankel (1957), using commercially available kits and according to Sigma Technical Bulletin 505.[1] Serum glutamic pyruvic transaminase catalyzes the reversible reaction between alanine with alpha-ketoglutaric acid and glutamic acid with pyruvic acid. The

---

[1]Sigma Chemical Company, St. Louis, Missouri.

reaction is measured colorimetrically at 505 mμ by the reddish-brown hydrazone which pyruvic acid forms with 2,4-dinitrophenylhydrazone in alkaline solution. Assay results were reported in Sigma-Frankel units. A theoretical Sigma-Frankel unit of SGPT or SGOT is the enzyme activity which will form 4.82×10^{-4} micromoles of glutamate per minute at 25 C and pH of 7.5.

Serum glutamic oxaloacetic transaminase

Determination of serum levels of this enzyme were carried out by the same method as used to assay SGPT. One major difference was that a substrate containing aspartic acid and alpha-ketoglutaric acid was used for assaying GOT while an alanine and alpha-ketoglutaric acid containing substrate was used for determining SGPT levels. Glutamic oxaloacetic transaminase reversibly catalyzes the reaction between aspartic acid plus alpha-ketoglutaric acid and glutamic acid plus oxaloacetic acid. The reaction is measured, in this technique, by the reddish-brown hydrazone which oxaloacetic acid forms with 2,4-dinitrophenylhydrazone at an alkaline pH. Results were reported in Sigma Frankel units.

Serum lactic dehydrogenase

Serum levels of LDH were determined by the colorimetric method of Cabaud et al. (1958). Commercially available reagent kits were used as described in Sigma Technical Bulletin

No. 500.[1] Lactic dehydrogenase is an enzyme which catalyzes the reversible reaction between pyruvic acid and reduced nicotinamide adenine dinucleotide (NADH) to or from lactic acid and NAD. The procedure employed measures enzyme concentration by reacting unchanged pyruvic acid with 2,4-dinitrophenylhydrazine, forming an intensely colored hydrazone with a maximal optical density (O.D.) in the wavelength range of 400-550 mμ. Lactic acid, reduced NAD, and NAD do not contribute a significant O.D. Consequently, the amount of pyruvate remaining after incubation is inversely proportional to the amount of LDH present in the reaction. Results were reported in Berger-Broida (B-B) units. One B-B unit of LDH activity will reduce 4.8×10^{-4} micromoles of pyruvate per minute at 25 C.

Experimental Animals

All pigs used in these experiments were from closed herds originally stocked with pigs derived from caesarean section. Groups 1, 3 and 4 were from the swine herd at the NADL, Ames, Iowa. This herd has been maintained in isolation for 9 years, and no outbreaks of HC have occurred during that time. Clinical, virologic, immunofluorescent tissue section, histologic and postmortem examinations of several thousand pigs from this herd have shown no evidence of HC. Group 2 consisted of

---

[1]Sigma Chemical Company, St. Louis, Missouri.

5 Hampshire barrows, 10 weeks of age and weighing between 25 to 35 lbs., obtained from an independent swine producer. This herd was also stocked from caesarean section derived animals and was maintained under conditions similar to those of the NADL swine herd. All animals were fed NADL Pig Grower 539,[1] a nutritionally complete and balanced ration (National Academy of Sciences, 1968) containing no arsenicals, antibiotics, or other drug additives.

Fifty swine which were bled for ESR determinations were from the NADL swine herd. The characteristics of this herd are described in Table 2.

Hog Cholera Viruses

The two hog cholera viruses designated HCV-325 and HCV-11325 are also referred to as the virulent and moderately virulent HCV's respectively.

HCV-325

A highly lethal strain of HCV which has been maintained by serial passage in pigs. It is commonly referred to as the Ames challenge strain or Ames 325 strain of HCV. Normally, this strain of virus will kill between 70 and 100 percent of susceptible pigs infected by intramuscular injection of 2-3 ml. of virus-containing blood from previously infected animals.

---

[1]United Suppliers, Inc., Eldora, Iowa.

HCV-11325

A strain of HCV isolated from an outbreak of HC in Maryland. Fluorescent antibody tissue culture tests failed to detect this virus, however swine blood buffy coat cell cultures did detect its presence (Kresse[1]). Inoculation of 3 ml. of splenic tissue suspension from the initially infected pigs into a specific-pathogen-free (SPF) pig produced a mild case of HC which persisted for 114 days. Three ml. of blood collected from this animal during the early stage of illness was inoculated intramuscularly into another animal. Within 5 days following inoculation the animal refused to eat and was still inappetent eleven days postinfection. Blood was collected from the vena cava, defibrinated and frozen at -20 C. This was the source of inoculum in these studies. Euthanasia was performed the same day and a complete necropsy conducted. Both FATS and FATC examinations confirmed the presence of HCV. Tonsillar erosions and pulmonary and splenic infarcts were present. This strain of virus was graciously supplied by Dr. W. C. Stewart of the Virology Section of Diagnostic Services, Animal Health Division, NADL, Ames, Iowa.

---

[1]Kresse, J. W., Virology Section, Diagnostic Services, NADL, Ames, Iowa. Preliminary studies on the use of swine buffy coat cell cultures for isolation of HCV. Private communication. 1969.

Experimental Procedure

Trial 1

Five Yorkshire and Chester White crossbred pigs, 9 weeks of age were placed in isolation. Control levels were established by determining values for the ESR, PCV, Hgb, TWBC, TRBC, differential WBC count, bone marrow cell count, reticulocyte count, total serum protein, serum protein electrophoretic distribution, SAP, SAcP, SGPT, SGOT, and LDH, for 4 days. After samples were collected on the fourth day, the pigs were infected by intramuscular injection of 3 ml. of defibrinated swine blood containing HCV-325, a virulent strain of HCV. Blood and bone marrow were collected prior to infection and on each consecutive day until the animals died, became moribund or the trial was terminated by euthanasia of remaining animals. Tonsillar biopsies were collected at intervals of time selected to provide the most meaningful results when correlated with results of bone marrow smears examined by the FATST.

Trial 2

Five Hampshire pigs, 10 weeks of age were placed in isolation. Control levels were established for the various measurements as in Trial 1. The animals were infected by intramuscular injection of 3 ml. of defibrinated swine blood containing HCV-11325, a moderately virulent strain of HCV. Bone marrow and blood samples were collected prior to infection

and every other day, excluding weekends, until the animals died, became moribund or were euthanatized. Tonsil biopsies were collected in the same manner as for Trial 1.

Trial 3

This was a duplication of Trial 1. The animals and viral strain were similar.

Trial 4

This trial was largely a duplication of Trial 2. One exception was that the pigs were of both sexes, whereas group 2 consisted of barrows. Swine in group 2 were of the Hampshire breed, while those in this trial were Yorkshire and Chester White crossbred animals.

Biometrical Procedures

Comparisons were made between group data collected before exposure and on individual days after exposure with a t-test using a pooled estimate of variance. The pooled estimate of variance was obtained from an analysis of variance in which pigs were used as blocks.

RESULTS

Hematologic Study Results

Erythrocyte sedimentation rate

Method comparison study Erythrocyte sedimentation rates were determined on blood samples from 50 pigs. The age, weight, sex, breed and specific-pathogen-free status of the animals are listed in Table 2. The ESR of each blood sample was determined in triplicate by each of 3 methods: modified Westergren method using diluted blood, Westergren method with undiluted blood and the Wintrobe method using undiluted blood. Results of these determinations are in Table 3. The consistency, or precision in statistical terms, of the observations within the 50 animals was evaluated with several criteria. These included average range, average variance, average coefficient of variation and pooled variance, as shown in Table 4. Results of all evaluating criteria indicate that the modified Westergren was the most consistent method for determining the ESR of swine blood in this study. All ESR measurements were made by this method during the study. Thirty-four young pigs, 1 to 2 months of age and of both sexes, had ESR rates with a mean and standard error of 2.81 ($\pm$ 3.79) mm./hr. There was no significant difference in the sedimentation rate between sexes in this group. Average ESR for 13, 3-month-old, female pigs was 2.36 ($\pm$ 0.15) mm./hr. and for 3 males, 6 to 6 and one-half-months-old, the average ESR was 6.83 mm./hr.

Table 2. Characteristics of swine used for comparison of ESR methods

No.	Weight	Age	Sex	Breed[a]	SPF
1		2 mo.	M	Land & Hamp	
2		2 mo.	M	Land & Hamp	
3	185	6 1/2 mo.	CM		
4	165	6 1/2 mo.	CM		
5	165	6 mo.	CM		
6		3 1/3 mo.	M	Land & Hamp	2 gen.
7	65	3 mo.	F	7/8 York	2 gen.
8	62	3 mo.	F	7/8 York	2 gen.
9	58	3 mo.	F	7/8 York	2 gen.
10	57	3 mo.	F	7/8 York	2 gen.
11	72	3 mo.	F	7/8 York	2 gen.
12	90	3 mo.	F	7/8 York	2 gen.
13	73	3 mo.	F	7/8 York	2 gen.
14	82	3 mo.	F	7/8 York	2 gen.
15	82	3 mo.	F	7/8 York	2 gen.
16	72	3 mo.	F	7/8 York	2 gen.
17	64	3 mo.	F	7/8 York	2 gen.
18	26	1 mo.	F	7/8 York	2 gen.
19	24	1 mo.	F	7/8 York	2 gen.
20	22	1 mo.	F	7/8 York	2 gen.
21	25	1 mo.	F	7/8 York	2 gen.
22	20	1 mo.	M	7/8 York	2 gen.
23	23	1 mo.	F	7/8 York	2 gen.
24	26	1 mo.	F	7/8 York	2 gen.
25	26	1 mo.	M	7/8 York	2 gen.

[a]Land=Landrace, Hamp=Hampshire, York=Yorkshire.

Table 2 (Continued)

No.	Weight	Age	Sex	Breed	SPF
26	23	1 mo.	M	7/8 York	2 gen.
27	22 1/2	1 mo.	M	7/8 York	2 gen.
28	22	1 mo.	M	7/8 York	2 gen.
29	24	1 mo.	F	7/8 York	2 gen.
30	16	1 mo.	F	7/8 York	2 gen.
31	21	1 mo.	F	York	2 gen.
32	21	1 mo.	F	York	2 gen.
33	19	1 mo.	F	York	2 gen.
34	22	1 mo.	M	York	2 gen.
35	23	1 mo.	F	York	2 gen.
36	21	1 mo.	M	York	2 gen.
37	23	1 mo.	F	York	2 gen.
38	25	1 mo.	M	York	2 gen.
39	16	1 mo.	F	York	2 gen.
40	31	1 mo.	M	7/8 York	2 gen.
41	33	1 mo.	F	7/8 York	2 gen.
42	29	1 mo.	M	7/8 York	2 gen.
43	28	1 mo.	M	7/8 York	2 gen.
44	25	1 mo.	M	7/8 York	2 gen.
45	26	1 mo.	M	7/8 York	2 gen.
46	31	1 mo.	F	7/8 York	2 gen.
47	30	1 1/2 mo.	M	7/8 York	2 gen.
48	25	1 1/2 mo.	F	York	2 gen.
49	33	1 1/2 mo.	F	York	2 gen.
50	34	1 1/2 mo.	M	York	2 gen.

Table 3. Comparison of three erythrocyte sedimentation rate methods on swine blood[a]

No.	Westergren undiluted					Westergren diluted					Wintrobe				
	1	2	3	$\bar{X}$[b]	R[c]	1	2	3	$\bar{X}$	R	1	2	3	$\bar{X}$	R
1	2.5	2.5	1.0	2.0	1.5	1.5	1.5	1.5	1.5	0.0	1.5	2.0	2.0	1.8	0.5
2	5.5	5.5	5.0	5.3	0.5	3.3	3.5	4.0	3.6	0.8	5.0	2.0	3.0	3.3	3.0
3	2.5	8.0	6.0	5.5	5.5	6.5	6.0	7.0	6.5	1.0	9.5	11.0	9.5	10.0	1.5
4	7.0	12.0	13.0	10.7	6.0	7.0	6.0	7.0	6.7	1.0	11.0	9.5	12.0	10.8	2.5
5	8.5	6.5	1.5	5.5	7.0	7.0	8.0	7.0	7.3	1.0	8.5	6.5	6.5	7.2	2.0
6	1.5	0.5	0.5	0.8	1.0†	1.0	1.0	i.s.[d]	1.0	0.0	1.5	2.5	1.0	1.7	1.5
7	7.0	8.0	8.0	7.7	1.0	4.5	4.0	4.0	4.2	0.5	7.0	6.0	7.0	6.7	1.0
8	6.0	6.0	6.5	6.2	0.5	4.5	5.0	4.5	4.7	0.5	7.0	7.0	6.5	6.8	0.5
9	3.0	3.0	3.0	3.0	0.0	1.5	1.5	1.5	1.5	0.0	2.5	3.0	2.5	2.7	0.5
10	6.0	7.0	7.5	6.8	1.5	5.0	5.5	5.0	5.2	0.5	7.0	7.0	7.0	7.0	0.0
11	2.0	2.0	2.0	2.0	0.0	1.5	1.5	1.5	1.5	0.0	0.5	0.5	0.5	0.5	0.0
12	5.0	4.0	5.0	4.7	1.0	3.0	3.0	3.0	3.0	0.0	3.5	3.0	4.0	3.5	1.0
13	5.0	5.0	5.0	5.0	0.0	3.0	3.0	3.0	3.0	0.0	3.5	4.0	4.5	4.0	1.0
14	3.0	3.0	3.0	3.0	0.0	1.5	1.5	1.5	1.5	0.0	2.0	2.0	2.0	2.0	0.0
15	1.0	1.5	1.0	1.2	0.5	1.0	1.0	1.0	1.0	0.0	0.5	1.0	1.0	0.8	0.5
16	1.5	2.0	2.0	1.8	0.5	1.0	1.0	1.0	1.0	0.0	1.0	2.0	1.5	1.5	1.0
17	1.5	2.0	2.5	2.0	1.0	1.5	1.5	1.5	1.5	0.0	1.5	1.5	1.0	1.3	0.5
18	3.0	3.0	2.5	2.8	0.5	2.0	2.0	2.5	2.2	0.5	2.0	2.5	2.0	2.2	0.5
19	2.0	1.0	2.0	1.7	1.0	3.0	3.0	3.0	3.0	0.0	1.5	0.5	1.0	1.0	1.0
20	5.0	3.0	4.5	4.2	2.0	3.0	3.0	3.0	3.0	0.0	3.0	3.0	3.0	3.0	0.0
21	6.0	6.5	6.5	6.3	0.5	4.0	4.0	3.5	3.8	0.5	6.0	5.5	6.0	5.8	0.5
22	2.0	2.0	2.0	2.0	0.0	3.0	3.5	3.5	3.3	0.5	1.0	1.0	1.5	1.2	0.5

[a]Erythrocyte sedimentation rate results in mm./hr.

[b]$\bar{X}$ - mean of the three sample values.

[c]R - range of the three sample values.

[d]i.s. - insufficient sample.

Table 3 (Continued)

	Westergren undiluted					Westergren diluted					Wintrobe				
No.	1	2	3	$\bar{X}$	R	1	2	3	$\bar{X}$	R	1	2	3	$\bar{X}$	R
23	3.0	4.0	3.0	3.3	1.0	2.0	2.0	2.0	2.0	0.0	1.5	1.0	1.0	1.2	0.5
24	2.0	2.0	2.0	2.0	0.0	2.0	2.5	2.0	2.2	0.5	1.0	1.0	1.0	1.0	0.0
25	2.0	3.0	2.5	2.5	1.0	1.5	1.5	1.5	1.5	0.0	1.0	1.5	1.0	1.2	0.5
26	6.0	6.5	7.0	6.5	1.0	3.5	4.0	4.0	3.8	0.5	5.0	5.5	5.0	5.2	0.5
27	5.0	7.0	6.0	6.0	2.0	4.0	4.0	4.0	4.0	0.0	5.5	5.5	4.0	5.0	1.5
28	1.5	2.0	2.0	1.8	0.5	1.0	1.5	1.5	1.3	0.5	0.5	0.5	1.0	0.7	0.5
29	2.0	2.0	1.5	1.8	0.5	1.5	1.5	1.5	1.5	0.0	1.0	1.0	1.0	1.0	0.0
30	1.5	1.0	1.5	1.3	0.5	0.5	0.5	1.0	0.7	0.5	1.0	0.5	0.5	0.7	0.5
31	2.0	2.0	2.0	2.0	0.0	1.0	1.0	1.0	1.0	0.0	1.0	0.5	1.0	0.8	0.5
32	2.0	2.0	2.0	2.0	0.0	1.0	1.0	1.0	1.0	0.0	1.5	2.0	1.0	1.5	1.0
33	1.0	1.0	0.5	0.8	0.5	0.5	0.5	0.5	0.5	0.0	0.5	0.5	0.0	0.3	0.5
34	3.0	2.5	3.0	2.8	0.5	2.0	2.0	2.0	2.0	0.0	2.0	2.0	1.5	1.8	0.5
35	0.5	1.0	0.5	0.7	0.5	0.5	0.5	0.5	0.5	0.0	0.0	0.0	0.0	0.0	0.0
36	1.5	1.0	1.5	1.3	0.5	1.0	1.0	1.0	1.0	0.0	1.5	1.0	1.5	1.3	0.5
37	1.5	2.0	1.5	1.7	0.5	1.0	1.0	0.5	0.8	0.5	1.0	1.5	1.0	1.2	0.5
38	3.0	2.5	3.0	2.8	0.5	2.0	2.0	1.5	1.8	0.5	1.5	2.5	2.0	2.0	1.0
39	3.0	3.5	3.0	3.2	0.5	2.5	2.0	2.0	2.2	0.5	2.0	3.0	3.0	2.7	1.0
40	29.0	30.0	50.0	36.3	21.0	21.0	21.0	24.0	22.0	3.0	33.0	33.5	32.0	32.8	1.5
41	0.5	0.5	0.5	0.5	0.0	1.0	1.0	1.0	1.0	0.0	0.5	0.5	0.5	0.5	0.0
42	10.5	10.0	10.0	10.2	0.5	7.0	7.5	7.5	7.3	0.5	9.0	9.0	9.0	9.0	0.0
43	2.5	2.5	2.5	2.5	0.0	1.0	1.0	1.0	1.0	0.0	1.5	1.5	1.5	1.5	0.0
44	1.0	0.5	0.5	0.7	0.5	1.0	1.0	1.0	1.0	0.0	0.0	0.5	0.5	0.3	0.5
45	2.0	2.5	3.0	2.5	1.0	1.5	1.5	1.5	1.5	0.0	2.5	2.0	2.5	2.3	0.5
46	2.0	1.5	1.5	1.7	0.5	1.5	1.5	1.5	1.5	0.0	1.5	1.0	1.5	1.3	0.5
47	4.0	4.0	3.0	3.7	1.0	2.5	2.5	2.5	2.5	0.0	4.0	3.0	3.5	3.5	1.0
48	3.0	2.5	2.0	2.5	1.0	2.0	2.0	2.5	2.2	0.5	3.0	3.0	4.0	3.3	1.0
49	2.0	3.0	2.5	2.5	1.0	1.5	2.0	2.0	1.8	0.5	2.0	2.5	2.5	2.3	0.5
50	6.5	6.0	6.5	6.3	0.5	4.0	5.0	4.5	4.5	1.0	6.0	6.5	6.0	6.2	0.5

Table 4. Consistency of ESR determinations on blood samples from 50 pigs

	Westergren undiluted	Westergren diluted	Wintrobe
Average range	1.41	0.37	0.67
Average variance	3.65	0.16	0.23
Average coefficient of variation	0.15	0.06	0.18
Pooled variance	3.78	0.16	0.25

Modified Westergren versus dilute Wintrobe method

Since the modified Westergren procedure had not been used to determine the ESR in sick swine, it was necessary to compare it with the Wintrobe method for determining the ESR of sick pigs. Gambino et al. (1965), using human blood, pointed out that in ESR determinations, more precision and accuracy can be obtained with diluted blood using either the Westergren or Wintrobe tubes. In view of this, the Wintrobe method was conducted using diluted blood. Forty-eight hours after infection with HCV the first samples were collected and the sedimentation rates determined. Animals in the first group were bled daily while those in the second group were bled every other day. The ESR was determined as soon after collection of blood as possible. Table 5 contains results of this study. In the first group, the ESR increased from 2 to 6 times the normal

Table 5. Comparison of modified Westergren and dilute Wintrobe methods of determining the erythrocyte sedimentation rate of blood from swine infected 48 hours previously by a hog cholera virus of high virulence (Group I) and moderate virulence (Group II)[a]

	Group I									
	12825		12826		12827		12828		12829	
Date	Dilute West[b]	Dilute Win[c]	Dilute West	Dilute Win	Dilute West	Dilute Win	Dilute West	Dilute Win	Dilute West	Dilute Win
1969										
8-27	18.0	12.0	17.5	11.0	27.5	23.5	14.0	13.0	16.5	15.0
8-28	6.0	6.0	3.5	3.5	15.0	13.0	7.5	7.0	8.0	7.5
8-29	3.0	2.0	2.0	1.5	D[d]		1.0	1.5	6.5	6.0
8-30	6.5	5.5	2.5	3.0	D		D		11.5	9.5
8-31	11.0	9.0	D		D		D		18.0	14.5
9-1	12.0	10.5	D		D		D		17.0	12.5
9-2	12.5	13.5	D		D		D		18.5	14.5
9-3	15.5	15.0	D		D		D		20.0	15.5

[a]Erythrocyte sedimentation results in mm./hr.

[b]Modified Westergren.

[c]Wintrobe.

[d]D-animals died.

Table 5 (Continued)

	Group II									
	12941		12942		12943		12944		12945	
Date	Dilute West	Dilute Win	Dilute West	Dilute Win	Dilute West	Dilute Win	Dilute West	Dilute Win	Dilute West	Dilute Win
1969										
9-17	3.0	3.0	5.0	5.5	5.5	6.0	7.0	6.5	2.0	2.0
9-19	4.0	4.0	29.0	26.0	18.0	16.0	38.5	29.5	8.0	7.5
9-22	4.5	4.0	41.0	32.5	22.0	18.0	24.0	19.5	11.0	10.5
9-24	5.0	5.5	28.0	26.0	30.0	25.5	34.0	26.0	13.0	11.0
9-26	14.0	12.0	27.5	25.5	30.0	26.5	38.5	31.0	10.5	8.5
9-29	31.0	23.0	13.5	15.0	29.5	24.0	34.0	26.5	28.0	20.5
10-1	40.0	31.0	9.0	10.0	13.0	12.0	27.5	21.5	26.5	18.0
10-3	33.5	28.0	D		4.0	5.0	28.0	21.5	24.0	19.0
10-6	24.0	20.0	D		D		35.0	26.0	19.0	14.0
10-8	31.5	27.0	D		D		63.0	43.0	24.5	17.0
10-10	44.5	34.0	D		D		D		14.0	11.5
10-13	97.5	56.0	D		D		D		8.0	6.0
10-15	61.5	43.5	D		D		D		5.5	5.0

rate within 24 hours after infection. Total leukocyte counts decreased to approximately one-third or one-half their normal values, however only 2 of the 5 animals had TWBC counts which were considered leukopenic. Body temperatures also increased, within 24 hours after infection, to approximately 105.8 F. The second group of pigs, which had been infected with a less virulent strain of HC, reacted more slowly; however, the ESR increase occurred at the same time as the body temperature rose and the leukopenia developed. These results indicate that the modified Westergren and Wintrobe methods demonstrated changes in the ESR, produced by the HCV, as quickly as changes in body temperature and total leukocyte count. A comparison of the data on ESR, TWBC and body temperature values clearly demonstrates that the ESR demonstrated a pathologic change in the blood as definitely and in some cases more rapidly than the other two diagnostic criteria of HC.

Erythrocyte sedimentation rate of experimental animals

This series of observations is listed in Table 6. All of the animals infected with virulent HCV-325 had at least a two-fold increase in their ESR values by 24 hours after infection. This markedly increased rate persisted for 2 to 4 days and then declined sharply. Erythrocyte sedimentation rates of the 2 groups of animals (Groups II and IV) infected with the less virulent HCV-11325 strain also had elevated ESR values. However, a two-fold increase in rates was not evident for 2 to 5 days following infection. In contrast to the short period

Table 6. Changes in erythrocyte sedimentation rate produced by infection of susceptible pigs with highly virulent (HCV-325) and less virulent (HCV-11325) hog cholera viruses[a]

Date	Group I HCV-325 Animal numbers				
1969	12825	12826	12827	12828	12829
8-20	1.0	1.5	5.0	6.0	1.0
8-21	2.5	2.0	7.0	6.0	2.0
8-22[b]	3.0	3.5	9.5	5.0	3.0
8-25[b]	2.5	2.5	12.0	3.5	2.5
8-26	9.0	10.0	27.0	10.0	16.0
8-27	18.0	17.5	27.5	14.0	16.5
8-28	6.0	3.5	15.0	7.5	8.0
8-29	3.0	2.0	D[c]	1.0	6.5
8-30	6.5	2.5	D	D	11.5
8-31	11.0	D	D	D	18.0
9-1	12.0	D	D	D	17.0
9-2	15.5	D	D	D	18.5
9-3	15.5	D	D	D	20.0
	Group II HCV-11325				
	12941	12942	12943	12944	12945
9-10	2.0	4.5	2.5	3.0	2.5
9-11	4.5	4.0	3.0	4.0	3.0
9-12[b]	4.0	5.5	3.5	5.0	4.0
9-15[b]	3.5	4.5	3.5	3.5	2.5
9-17	3.0	5.0	5.5	7.0	2.0
9-19	4.0	29.0	18.0	38.5	8.0
9-22	4.5	41.0	22.0	24.0	11.0
9-24	5.0	28.0	30.0	34.0	13.0
9-26	14.0	27.5	30.0	38.5	10.5
9-29	31.0	13.5	29.5	34.0	28.0
10-1	40.0	9.0	13.0	27.5	26.5
10-3	33.5	D	4.0	28.0	24.0
10-6	24.0	D	D	35.0	19.0
10-8	31.5	D	D	63.0	24.5
10-10	44.5	D	D	D	14.0
10-13	97.5	D	D	D	8.0
10-15	61.5	D	D	D	5.5

[a]Erythrocyte sedimentation rate results in mm./hr.

[b]Date of infection.

[c]D-animal died.

Table 6 (Continued)

Date	Group III HCV-325 Animal numbers				
1969	13136	13137	13138	13139	13140
10-22	3.0	4.0	4.0	3.5	4.0
10-23	3.0	7.0	4.5	6.0	5.0
10-24	4.0	7.5	4.0	5.5	5.0
10-27	3.5	4.0	3.0	5.0	4.0
10-28	17.5	22.5	20.5	16.0	19.0
10-29	23.0	15.0	24.0	28.0	14.0
10-30	14.0	5.0	3.0	3.5	7.0
10-31	11.0	4.5	3.0	3.5	11.0
11-1	8.0	14.0	7.0	D	14.0
11-2	23.0	30.0	13.5	D	21.5
11-3	22.5	36.0	16.0	D	22.0
11-4	31.0	31.5	25.0	D	21.0
11-5	27.5	35.0	D	D	18.0
11-6	D	37.0	D	D	D
11-7	D	36.5	D	D	D

	Group IV HCV-11325				
	13023	13024	13143	13144	13145
11-12	3.0	3.0	2.5	3.5	2.5
11-13	4.0	4.0	2.0	4.5	3.5
11-14[b]	3.0	3.0	2.0	5.0	3.0
11-17[b]	3.0	3.0	2.0	4.5	4.5
11-19	5.0	5.0	4.5	6.0	5.5
11-21	20.0	24.5	20.0	28.0	27.5
11-24	29.0	17.5	31.0	57.5	19.5
11-26	17.5	15.0	22.0	67.0	14.0
11-28	25.5	24.5	21.0	63.0	14.0
12-1	D	16.0	55.0	D	19.5
12-3	D	30.0	30.0	D	19.0
12-5	D	25.0	D	D	14.0
12-8	D	21.5	D	D	5.0
12-10	D	19.0	D	D	5.0
12-12	D	23.5	D	D	5.0

during which the elevated ESR values persisted in the pigs infected by the virulent HCV, the rate in these animals remained increased to at least twice their normal levels for from 5 to 11 days.

Seven of ten animals (Groups I and III) infected with virulent HCV had abnormally high ESR values at the time of death or euthanasia. Eight of ten pigs infected with the moderately virulent HCV-11325 also had markedly increased ESR's at the time of death or conclusion of the respective study. In all cases, except those animals which died too early to manifest it, the ESR increases occurred in two periods separated by a period of decline of which was variable duration and intensity.

Statistically significant differences occurred between postinfection group means and pre-exposure group means on the days on which at least 3-fold increases of ESR occurred.

Packed cell volume

The sharp reduction in the PCV of pigs infected with highly virulent HCV-325 was quite dramatic. Within 24 hours after infection, PCV values of animals in Groups I and III dropped an average of 5.35 percent per animal. These data are listed in Table 7, which also contains PCV findings for the animals infected with the less virulent HCV-11325. An average drop of 3.0 percent per animal was observed in these animals during the initial 48 hours following infection.

Table 7. Changes in packed cell volume of young swine infected with highly virulent (HCV-325) or less virulent (HCV-11325) hog cholera virus[a]

Date	Group I HCV-325 Animal numbers				
1969	12825	12826	12827	12828	12829
8-20	33.5	35.0	34.5	34.5	37.0
8-21	32.5	34.5	33.0	37.0	36.5
8-22	33.5	35.0	31.5	36.0	34.5
8-25[b]	33.0	34.5	35.0	35.0	36.0
8-26	30.5	30.5	28.5	31.5	29.0
8-27	27.5	31.5	22.5	28.5	29.5
8-28	31.0	32.5	30.5	32.0	32.0
8-29	30.5	36.0	D[c]	37.5	31.0
8-30	26.0	38.0	D	D	29.5
8-31	26.0	D	D	D	28.5
9-1	27.5	D	D	D	28.5
9-2	26.0	D	D	D	28.5
9-3	24.0	D	D	D	27.0
	Group II HCV-11325				
	12941	12942	12943	12944	12945
9-10	34.5	33.5	34.5	34.5	34.0
9-11	35.5	32.5	34.0	34.5	35.0
9-12	34.0	32.5	33.0	35.0	35.0
9-15[b]	38.0	37.0	36.5	39.0	38.0
9-17	38.5	35.5	31.5	33.5	37.0
9-19	37.5	33.0	30.5	31.5	34.5
9-22	39.5	32.0	31.5	31.0	34.0
9-24	36.5	34.5	31.5	30.5	33.0
9-26	33.5	36.5	30.0	31.5	32.5
9-29	33.5	34.5	31.0	28.0	28.0
10-1	34.5	35.5	32.0	26.0	30.5
10-3	32.5	D	33.5	25.0	28.5
10-6	33.0	D	D	22.5	32.0
10-8	30.5	D	D	22.0	33.5
10-10	30.5	D	D	D	31.0
10-13	28.5	D	D	D	30.5
10-15	30.0	D	D	D	32.0

[a]Packed cell volume reported as percentage.

[b]Date of infection.

[c]D-animal died.

Table 7 (Continued)

Date	Group III HCV-325 Animal numbers				
1969	13136	13137	13138	13139	13140
10-22	36.5	36.5	36.0	36.5	35.0
10-23	35.5	35.0	36.0	36.0	31.5
10-24[b]	37.0	35.0	35.5	35.5	32.5
10-27[b]	37.5	36.5	36.0	36.0	34.5
10-28	32.5	29.5	30.5	30.5	27.5
10-29	28.0	30.5	30.5	30.0	28.5
10-30	29.5	31.5	31.5	37.0	29.0
10-31	32.0	33.5	31.5	40.5	28.5
11-1	30.5	31.5	31.5	D	28.0
11-2	29.5	29.5	31.0	D	26.5
11-3	29.0	28.5	31.0	D	27.5
11-4	29.5	28.0	28.0	D	26.5
11-5	28.5	27.0	D	D	27.0
11-6	D	26.5	D	D	D
11-7	D	27.5	D	D	D
	Group IV HCV-11325				
	13023	13024	13143	13144	13145
11-12	37.5	34.5	36.5	35.0	35.5
11-13	36.0	32.0	37.0	31.5	33.0
11-14[b]	35.0	32.0	36.5	31.5	33.0
11-17[b]	36.0	33.5	37.5	34.5	34.5
11-19	32.5	30.5	32.0	30.0	33.0
11-21	31.5	26.0	32.0	26.5	29.5
11-24	35.5	29.0	34.5	27.5	28.0
11-26	36.0	32.0	33.5	27.0	29.0
11-28	34.5	31.0	33.0	26.0	30.0
12-1	D	30.5	15.5	D	30.0
12-3	D	27.5	8.5	D	28.5
12-5	D	26.0	D	D	26.0
12-8	D	24.0	D	D	28.5
12-10	D	24.0	D	D	30.5
12-12	D	23.5	D	D	28.5

Following this initial, rapid reduction, the PCV of all animals continued to decrease. This reduction, although frequently irregular and occasionally reversed, persisted in 15 of the 20 animals until shortly before they died or the experiment was terminated. In some cases the PCV increased sharply for varying periods of time. In some cases the levels observed in comatose and moribund animals exceeded pre-infection levels. In both relatively healthy-appearing but sick animals and those which were moribund, the PCV, Hgb and TRBC followed similar response patterns to the effects of the disease.

Significant differences were observed in the mean values of Group I on days 1 through 3 and 5 through 7, Group II on days 14 through 28, Group III on days 0 through 10, and Group IV on days 14 through 23.

Hemoglobin concentration

A precipitous drop in Hgb occurred shortly after infection in animals infected by both strains of HCV (Table 8). More profound decreases in Hgb were observed in animals infected with virulent HCV-325. An average reduction in Hgb of 1.82 grams percent per animal occurred. The ten other animals infected with less virulent HCV-11325 had an average reduction of 0.88 grams percent per animal at 48 hours postinfection (p.i.). Transient elevations in Hgb levels occurred 2 to 7 days after the initial decline and varied in duration from 2 to 5 days. Elevations in Hgb were observed in animals which

Table 8. Influence of infection by hog cholera viruses of high (HCV-325) and moderate (HCV-11325) virulence on hemoglobin levels of young pigs[a]

Date	Group I HCV-325 Animal numbers				
1969	12825	12826	12827	12828	12829
8-20	10.3	11.5	11.2	11.7	12.4
8-21	10.2	11.4	10.3	12.0	12.4
8-22[b]	10.6	11.4	10.2	11.9	11.5
8-25[b]	10.0	11.2	10.7	11.4	12.0
8-26	9.4	9.9	8.1	10.6	9.6
8-27	8.7	10.4	6.8	9.4	9.0
8-28	9.6	10.6	10.0[c]	10.2	10.4
8-29	9.9	11.9	D[c]	11.9	10.3
8-30	9.4	11.7	D	D	10.2
8-31	8.3	D	D	D	9.3
9-1	8.4	D	D	D	9.6
9-2	7.7	D	D	D	9.0
9-3	7.2	D	D	D	8.3
	Group II HCV-11325				
	12941	12942	12943	12944	12945
9-10	10.9	10.4	10.6	11.0	10.9
9-11	11.0	10.3	10.4	10.7	11.0
9-12[b]	10.6	10.1	9.8	11.0	10.9
9-15[b]	11.8	11.5	10.9	12.2	12.0
9-17	12.6	10.7	9.5	10.3	11.9
9-19	11.6	10.2	9.2	9.6	11.2
9-22	12.2	10.1	9.2	9.4	10.7
9-24	11.5	10.9	9.4	9.2	10.3
9-26	10.7	11.5	8.8	9.7	10.1
9-29	10.6	10.7	9.1	8.3	8.7
10-1	11.0	11.2	9.2	8.0	9.5
10-3	10.4	D	9.7	7.5	8.7
10-6	10.4	D	D	7.0	10.1
10-8	9.6	D	D	6.6	10.7
10-10	9.6	D	D	D	9.9
10-13	8.8	D	D	D	9.5
10-15	9.1	D	D	D	9.8

[a]Hemoglobin reported in gm./100 ml.

[b]Date of infection.

[c]D-animal died.

Table 8 (Continued)

Date	Group III HCV-325 Animal numbers				
1969	13136	13137	13138	13139	13140
10-22	11.4	11.4	11.0	11.5	10.9
10-23	11.2	10.9	10.9	11.4	9.9
10-24[b]	11.5	10.7	10.9	11.2	10.4
10-27[b]	12.4	11.9	11.2	11.8	11.0
10-28	10.2	9.0	10.0	9.8	8.8
10-29	8.9	9.9	8.7	9.5	9.1
10-30	9.6	10.3	10.0	12.2	9.6
10-31	10.2	10.6	9.7	13.4	9.2
11-1	9.7	9.9	9.6	D	8.8
11-2	9.6	9.6	9.9	D	8.4
11-3	9.6	9.0	10.0	D	8.8
11-4	9.2	8.8	9.4	D	8.4
11-5	8.8	8.8	D	D	8.3
11-6	D	8.3	D	D	D
11-7	D	8.8	D	D	D

	Group IV HCV-11325				
	13023	13024	13143	13144	13145
11-12	12.0	10.9	11.9	11.1	11.4
11-13	11.5	10.2	11.9	10.0	10.3
11-14[b]	11.2	10.4	11.7	9.7	10.3
11-17[b]	11.4	10.6	12.4	11.0	10.9
11-19	10.3	9.8	10.7	9.7	10.4
11-21	10.4	8.3	10.3	8.0	9.4
11-24	11.4	9.1	11.2	9.0	9.0
11-26	11.5	10.4	10.9	8.3	9.1
11-28	10.7	9.7	10.6	8.1	9.1
12-1	D	9.7	4.5	D	9.0
12-3	D	8.4	2.1	D	8.8
12-5	D	7.9	D	D	7.9
12-8	D	7.6	D	D	8.8
12-10	D	7.5	D	D	9.4
12-12	D	7.2	D	D	8.8

were moribund at about the same time as the PCV increases were observed.

Total red blood cell numbers

Total erythrocyte numbers are recorded in Table 9. Decreases and transient increases in RBC numbers followed fluctuations of Hgb and PCV. The TRBC count of animals infected by HCV-325 dropped an average of 794,000 cells within 24 hours p.i., while TRBC counts in pigs inoculated with HCV-11325 were reduced an average of 713,000 within 48 hours p.i. After 48 hours p.i., the group of pigs infected by HCV-325 had TRBC counts averaging 996,000 less than at pre-inoculation levels. Erythrocyte numbers in the healthy young animals before infection were observed to fluctuate as much as 1 million RBC's from day to day.

Red blood cell indices

Three RBC-Hgb indices were calculated using results of determinations for PCV, Hgb and TRBC. They were mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin (MCH).

Mean corpuscular volume This is the average volume of each RBC and is calculated using the PCV and TRBC by the formula:

$$MCV = \frac{10 \times PCV}{TRBC \text{ number}} \text{ (in millions per cu. mm.)}$$

Results are reported in cubic microns.

Table 9. Red blood cell numbers of young pigs infected by a highly virulent (Groups I and III) and moderately virulent strain (Groups II and IV) of hog cholera virus[a]

Date	Group I HCV-325 Animal numbers				
1969	12825	12826	12827	12828	12829
8-20	720	620	803	669	863
8-21	661	564	700	659	642
8-22[b]	718	621	672	654	657
8-25[b]	688	574	715	549	653
8-26	613	518	621	536	572
8-27	663	590	475	526	552
8-28	674	594	669	593	618
8-29	627	650	D[c]	723	628
8-30	654	732	D	D	594
8-31	634	D	D	D	596
9-1	640	D	D	D	554
9-2	558	D	D	D	565
9-3	527	D	D	D	465
	Group II HCV-11325				
	12941	12942	12943	12944	12945
9-10	554	569	619	571	532
9-11	744	619	803	808	585
9-12[b]	536	667	627	708	514
9-15[b]	659	627	724	837	607
9-17	642	575	622	569	574
9-19	677	627	645	594	582
9-22	623	603	671	627	548
9-24	625	617	617	542	498
9-26	581	654	599	566	575
9-29	628	584	612	488	466
10-1	646	676	691	483	508
10-3	608	D	659	448	469
10-6	603	D	D	406	519
10-8	518	D	D	382	534
10-10	566	D	D	D	519
10-13	542	D	D	D	488
10-15	546	D	D	D	502

[a]Red blood cells reported as number of cells x 10^4/cu. mm.

[b]Date of infection.

[c]D-animal died.

Table 9 (Continued)

Date	Group III HCV-325 Animal numbers				
1969	13136	13137	13138	13139	13140
10-22	622	644	642	640	603
10-23	610	632	635	623	569
10-24[b]	657	604	647	639	578
10-27[b]	691	660	659	646	601
10-28	593	540	590	547	512
10-29	501	564	565	536	516
10-30	529	575	574	653	516
10-31	575	596	576	727	515
11-1	590	597	581	D	540
11-2	535	500	433	D	465
11-3	558	561	584	D	499
11-4	535	531	535	D	474
11-5	518	492	D	D	467
11-6	D	492	D	D	D
11-7	D	504	D	D	D
	Group IV HCV-11325				
	13023	13024	13143	13144	13145
11-12	632	556	603	565	594
11-13	585	501	590	524	549
11-14[b]	555	507	582	494	540
11-17[b]	594	538	606	571	548
11-19	537	499	549	505	524
11-21	542	428	542	430	518
11-24	599	484	585	480	496
11-26	658	558	584	464	510
11-28	560	520	543	448	527
12-1	D	516	263	D	510
12-3	D	440	129	D	482
12-5	D	426	D	D	456
12-8	D	419	D	D	508
12-10	D	400	D	D	529
12-12	D	383	D	D	509

Seventy-five percent of the animals manifested a reduction in this value during the course of the infection by both HCV strains. This was not a consistent and progressive change but was quite irregular (Table 10). The decrease was based on a comparison of initial and terminal results for this criterion. The largest increase was 15.2 cubic microns and biggest decrease was 9.7 cubic microns. There was no significant difference in this value between animals when the comparison is based on the virulence of the infecting HCV strain.

Mean corpuscular hemoglobin A calculated value representing the amount of Hgb in an average RBC. This value is calculated by the following formula:

$$MCH = \frac{\text{Hgb (gm./100 ml.) x 10}}{\text{TRBC number (millions per cu. mm.)}}$$

MCH values are reported in micromicrograms of Hgb.

There was no obvious change between initial and final results of this value among the pigs in the 4 groups (Table 11). Largest decrease observed was 3.5 percent and greatest increase was 3.4 percent.

Mean corpuscular hemoglobin concentration This is the hemoglobin concentration in an average erythrocyte. It is considered a ratio of weight of Hgb to the volume in which it is contained. Calculation is by the formula:

$$MCHC = \frac{\text{Hgb (gm./100 ml.) x 100}}{\text{PCV}}$$

Results are expressed as percent of RBC volume.

With one exception, there was little change in the MCHC

Table 10. Mean corpuscular volume of erythrocytes from young swine infected by virulent HCV-325 or less virulent HCV-11325 strains of hog cholera virus[a]

Date	Group I HCV-325 Animal numbers				
1969	12825	12826	12827	12828	12829
8-20	46.5	56.5	43.0	51.6	42.9
8-21	49.2	61.2	47.1	56.1	56.9
8-22[b]	46.5	56.4	46.9	55.0	52.5
8-25[b]	47.8	60.1	49.0	64.8	55.1
8-26	49.8	58.9	45.9	58.8	50.7
8-27	41.5	53.4	47.4	54.2	53.4
8-28	46.0	54.7	45.6	54.0	51.8
8-29	48.6	55.4	D[c]	51.9	49.4
8-30	39.8	51.9	D	D	49.7
8-31	41.0	D	D	D	47.8
9-1	43.0	D	D	D	51.4
9-2	46.6	D	D	D	50.4
9-3	45.5	D	D	D	58.1
	Group II HCV-11325				
	12941	12942	12943	12944	12945
9-10	62.3	58.9	55.7	60.4	63.9
9-11	47.7	52.5	42.3	42.7	59.8
9-12[b]	63.4	48.7	52.6	49.4	68.1
9-15[b]	57.7	59.0	50.4	46.6	62.6
9-17	60.0	61.7	50.6	58.9	64.5
9-19	55.4	52.6	47.3	53.0	59.3
9-22	63.4	53.1	46.9	49.4	62.0
9-24	58.4	55.9	51.1	56.3	66.3
9-26	57.7	55.8	50.1	55.7	56.5
9-29	53.3	59.1	50.7	57.4	60.1
10-1	53.4	52.5	46.3	53.8	60.0
10-3	53.5	D	50.8	55.8	60.8
10-6	54.7	D	D	55.4	61.7
10-8	58.9	D	D	57.6	62.7
10-10	53.9	D	D	D	59.7
10-13	52.6	D	D	D	62.5

[a]Mean corpuscular volume reported in cubic microns.

[b]Date of infection.

[c]D-animal died.

Table 10 (Continued)

Date	Group III HCV-325 Animal numbers				
1969	13136	13137	13138	13139	13140
10-22	58.7	56.7	56.1	57.0	58.0
10-23	58.2	55.4	56.7	57.8	55.4
10-24[b]	56.3	57.9	54.9	55.6	56.2
10-27[b]	54.3	55.3	54.6	55.7	57.4
10-28	54.8	54.6	51.7	55.8	53.7
10-29	55.9	54.1	54.0	56.0	55.2
10-30	55.8	54.8	54.9	56.7	56.2
10-31	55.7	56.2	54.7	55.7	55.3
11-1	51.7	52.8	54.2	D	51.9
11-2	55.1	59.0	71.6	D	57.0
11-3	52.0	50.8	53.1	D	55.1
11-4	55.1	52.7	52.3	D	55.9
11-5	55.0	54.9	D	D	57.8
11-6	D	53.9	D	D	D
11-7	D	54.6	D	D	D
	Group IV HCV-11325				
	13023	13024	13143	13144	13145
11-12	59.3	62.1	60.5	61.9	59.8
11-13	61.5	63.9	62.7	60.1	60.1
11-14[b]	63.1	63.1	62.7	63.8	61.1
11-17[b]	60.6	62.3	61.9	60.4	63.0
11-19	60.5	61.1	58.3	59.4	63.0
11-21	58.1	60.7	59.0	61.6	56.9
11-24	59.3	60.0	59.0	57.3	56.5
11-26	54.7	57.3	57.4	58.2	56.9
11-28	61.6	59.6	60.8	58.0	56.9
12-1	D	59.1	58.9	D	58.8
12-3	D	62.5	65.9	D	59.1
12-5	D	61.0	D	D	57.0
12-8	D	57.3	D	D	56.1
12-10	D	60.0	D	D	57.7
12-12	D	61.4	D	D	56.0

Table 11. Mean corpuscular hemoglobin of erythrocytes from young swine infected by virulent HCV-325 or less virulent HCV-11325 hog cholera virus[a]

Date	Group I HCV-325 Animal numbers				
1969	12825	12826	12827	12828	12829
8-20	14.3	18.5	13.9	17.5	14.4
8-21	15.4	20.2	14.7	18.2	19.3
8-22[b]	14.7	18.4	15.2	18.2	17.5
8-25[b]	14.5	19.5	15.0	20.8	18.4
8-26	15.3	19.1	13.0	19.8	16.8
8-27	13.1	17.6	14.3	17.9	16.3
8-28	14.2	17.8	14.9	17.2	16.8
8-29	15.8	18.3	D[c]	16.5	16.4
8-30	14.4	16.0	D	D	17.2
8-31	13.1	D	D	D	15.6
9-1	13.1	D	D	D	17.3
9-2	13.8	D	D	D	15.9
9-3	13.7	D	D	D	17.8
	Group II HCV-11325				
	12941	12942	12943	12944	12945
9-10	19.7	18.3	17.1	19.3	20.5
9-11	14.8	16.6	13.0	13.2	18.8
9-12[b]	19.8	15.1	15.6	15.5	21.2
9-15[b]	17.9	18.3	15.1	14.6	19.8
9-17	19.6	18.6	15.3	18.1	20.7
9-19	17.1	16.3	14.3	16.2	19.2
9-22	19.6	16.7	13.7	15.0	19.5
9-24	18.4	17.7	15.2	17.0	20.7
9-26	18.4	17.6	14.7	17.1	17.6
9-29	16.9	18.3	14.9	17.0	18.7
10-1	17.0	16.6	13.3	16.6	18.7
10-3	17.1	D	14.7	16.7	18.6
10-6	17.2	D	D	17.2	19.5
10-8	18.5	D	D	17.3	20.0
10-10	17.0	D	D	D	19.1
10-13	16.2	D	D	D	19.5

[a]Mean corpuscular hemoglobin reported in micromicrograms.

[b]Date of infection.

[c]D-animal died.

Table 11 (Continued)

Date	Group III HCV-325 Animal numbers				
1969	13136	13137	13138	13139	13140
10-22	18.3	17.7	17.1	18.0	18.1
10-23	18.4	17.2	17.2	18.3	17.4
10-24[b]	17.5	17.7	16.8	17.5	18.0
10-27[b]	17.9	18.0	17.0	18.3	18.3
10-28	17.2	16.7	16.9	17.9	17.2
10-29	17.8	17.6	15.4	17.7	17.6
10-30	18.1	17.9	17.4	18.7	18.6
10-31	17.7	17.8	16.8	18.4	17.9
11-1	16.4	16.6	16.5	D	16.3
11-2	17.9	19.2	22.9	D	18.1
11-3	17.2	16.0	17.1	D	17.6
11-4	17.2	16.6	17.6	D	17.7
11-5	17.0	17.9	D	D	17.8
11-6	D	16.9	D	D	D
11-7	D	17.5	D	D	D
	Group IV HCV-11325				
	13023	13024	13143	13144	13145
11-12	19.0	19.6	19.7	19.6	19.2
11-13	19.7	20.4	20.2	19.1	18.8
11-14[b]	20.2	20.5	20.1	19.6	19.1
11-17[b]	19.2	19.7	20.5	19.3	19.9
11-19	19.2	19.6	19.5	19.2	19.8
11-21	19.2	19.4	19.0	18.6	18.1
11-24	19.0	18.8	19.1	18.8	18.1
11-26	17.5	18.6	18.7	17.9	17.8
11-28	19.1	18.7	19.5	18.1	17.3
12-1	D	18.8	17.1	D	17.6
12-3	D	19.1	16.3	D	18.3
12-5	D	18.5	D	D	17.3
12-8	D	18.1	D	D	17.3
12-10	D	18.8	D	D	17.8
12-12	D	18.8	D	D	17.3

values of the pigs in all groups during the course of the disease. Animal 13143 had an initial MCHC of 32.6 and terminal report of 24.7 micromicrograms of Hgb. A majority of the other animals had changes of less than 3.5 micromicrograms of Hgb per animal. Results of this value fluctuated sharply, as did the MCV and MCH values, during the course of the disease (Table 12).

Total white blood cell counts

Data from TWBC counts are tabulated in Table 13. A severe drop in numbers of TWBC was observed, as expected, within 24 hours after infection with virulent strain HCV-325 (Groups I and III). Counts below 9,000 WBC per cu. mm. were observed in all pigs of Groups I and III within 48 hours p.i. and in 70 percent of these pigs TWBC counts of 9,000 or above were not detected after the initial fall. Three animals in Group III had counts above 9,000 on 1 or 2 days, after which a profound leukopenia prevailed. A TWBC count below 10,000 cells per cu. mm. is considered to be in the leukopenic range for swine of this age (Dunne, 1963). Among the animals of Groups II and IV infected with the less virulent HCV-11325, much longer periods of time between infection and onset of leukopenia were observed. In Group II, leukopenic levels were not observed until 144 hours p.i. and at that time only 1 of the 5 animals had a TWBC count in the leukopenic range. However all 5 animals in Group IV had leukocyte counts in the leuko-

Table 12. Mean corpuscular hemoglobin concentration of red blood cells from swine infected by either highly virulent HCV-325 or less virulent HCV-11325 hog cholera virus[a]

Date	Group I HCV-325 Animal numbers				
1969	12825	12826	12827	12828	12829
8-20	30.7	32.9	32.5	33.9	33.5
8-21	31.4	33.0	31.2	32.4	34.0
8-22[b]	31.6	32.6	32.4	33.1	33.3
8-25[b]	30.3	32.5	30.6	32.6	33.3
8-26	30.8	32.5	28.4	33.7	33.1
8-27	31.6	33.0	30.2	33.0	30.5
8-28	31.0	32.6	32.8	31.9	32.5
8-29	32.5	33.1	D[c]	31.7	33.2
8-30	36.2	30.8	D	D	34.6
8-31	31.9	D	D	D	32.6
9-1	30.5	D	D	D	33.7
9-2	29.6	D	D	D	31.6
9-3	30.0	D	D	D	30.7
	Group II HCV-11325				
	12941	12942	12943	12944	12945
9-10	31.6	31.0	30.7	31.9	32.1
9-11	31.0	31.7	30.6	31.0	31.4
9-12[b]	31.2	31.1	29.7	31.4	31.1
9-15[b]	31.1	31.1	29.9	31.3	31.6
9-17	32.7	30.1	30.2	30.7	32.2
9-19	30.9	30.9	30.2	30.5	32.5
9-22	30.9	31.6	29.2	30.3	31.5
9-24	31.5	31.6	29.8	30.2	31.2
9-26	31.9	31.5	29.3	30.8	31.1
9-29	31.6	31.0	29.4	29.6	31.1
10-1	31.9	31.5	28.8	30.8	31.1
10-3	32.0	D	29.0	30.0	30.5
10-6	31.5	D	D	31.1	31.6
10-8	31.5	D	D	30.0	31.9
10-10	31.5	D	D	D	31.9
10-13	30.9	D	D	D	31.1

[a]Mean corpuscular hemoglobin reported in percentage.

[b]Date of infection.

[c]D-animal died.

Table 12 (Continued)

Date	Group III HCV-325 Animal numbers				
1969	13136	13137	13138	13139	13140
10-22	31.2	31.2	30.6	31.5	31.1
10-23	31.5	31.1	30.3	31.7	31.4
10-24[b]	31.1	30.6	30.7	31.5	32.0
10-27[b]	33.1	32.6	31.1	32.8	31.9
10-28	31.4	30.5	32.8	32.1	32.0
10-29	31.8	32.5	28.5	31.7	31.9
10-30	32.5	32.7	31.7	33.0	33.1
10-31	31.9	31.6	30.8	33.1	32.3
11-1	31.8	31.4	30.5	D	31.4
11-2	32.5	32.5	31.9	D	31.7
11-3	33.1	31.6	32.3	D	32.0
11-4	31.2	31.4	33.6	D	31.7
11-5	30.9	32.6	D	D	30.7
11-6	D	31.3	D	D	D
11-7	D	32.0	D	D	D
	Group IV HCV-11325				
	13023	13024	13143	13144	13145
11-12	32.0	31.6	32.6	31.7	32.1
11-13	31.9	31.9	32.2	31.7	31.2
11-14[b]	32.0	32.5	32.1	30.8	31.2
11-17[b]	31.7	31.6	33.1	31.9	31.6
11-19	31.7	32.1	33.4	32.3	31.5
11-21	33.0	32.0	32.2	30.2	31.9
11-24	32.1	31.4	32.5	32.7	32.1
11-26	31.9	32.5	32.5	30.7	31.4
11-28	31.0	31.3	32.1	31.2	30.3
12-1	D	31.8	29.0	D	30.0
12-3	D	30.5	24.7	D	30.9
12-5	D	30.4	D	D	30.4
12-8	D	31.7	D	D	30.9
12-10	D	31.3	D	D	30.8
12-12	D	30.6	D	D	30.9

Table 13. Total white blood cell counts of young pigs infected by a highly virulent strain (HCV-325) and a less virulent strain (HCV-11325) of hog cholera virus[a]

Date	Group I HCV-325 Animal numbers				
1969	12825	12826	12827	12828	12829
8-20	21,582	19,303[b]	18,408[b]	20,500[b]	21,700[b]
8-21	32,836	23,500[b]	17,800[b]	19,800[b]	21,194[b]
8-22	28,557	30,149[b]	20,600[b]	19,800[b]	23,000[b]
8-25[c]	24,158	19,300[b]	17,900[b]	20,900[b]	25,800[b]
8-26	13,731	9,106[b]	13,069	12,900[b]	8,186[b]
8-27	8,684	5,461	6,714	5,369	4,577[b]
8-28	2,915	6,739	6,102[d]	7,429	5,181
8-29	4,436	7,349	D	8,143	3,836
8-30	7,929	8,522	D	D	5,403[b]
8-31	6,600[b]	D	D	D	7,222[b]
9-1	5,670[b]	D	D	D	7,208[b]
9-2	6,190[b]	D	D	D	7,390[b]
9-3	5,237[b]	D	D	D	5,171[b]
	Group II HCV-11325				
	12941	12942	12943	12944	12945
9-10	24,975[b]	28,500[b]	27,064[b]	18,905[b]	34,726[b]
9-11	25,100[b]	18,507[b]	26,700[b]	18,800[b]	35,800[b]
9-12[c]	25,300[b]	21,000[b]	25,000[b]	19,400[b]	31,400[b]
9-15	25,600[b]	21,100[b]	26,100[b]	34,500[b]	25,900[b]
9-17	31,243[b]	15,522	17,910[b]	20,800[b]	25,100[b]
9-19	32,100[b]	12,935	16,500[b]	13,831	12,700[b]
9-22	29,200[b]	13,930	12,600[b]	11,244[b]	8,663
9-24	25,100[b]	7,227[b]	7,590[b]	10,800[b]	9,603
9-26	14,900[b]	15,100[b]	8,375[b]	10,400[b]	7,590[b]
9-29	11,916[b]	8,500[b]	8,213[b]	7,790[b]	5,993[b]
10-1	7,638[b]	11,075[b]	10,141[b]	5,907[b]	8,396
10-3	11,048[b]	D	13,272	6,465[b]	10,869[b]
10-6	13,513[b]	D	D	6,299[b]	14,203[b]
10-8	9,349[b]	D	D	3,537[b]	21,293
10-10	5,958[b]	D	D	D	24,477[b]
10-13	6,336[b]	D	D	D	27,300[b]
10-15	8,214[b]	D	D	D	23,200[b]

[a]White blood cell counts reported in number of cells/cmm.

[b]No immature red blood cells observed in blood smear.

[c]Date of infection.

[d]D-animal died.

Table 13 (Continued)

Date	Group III HCV-325 Animal numbers				
1969	13136	13137	13138	13139	13140
10-22	25,700[b]	22,200[b]	25,400[b]	26,800[b]	20,500[b]
10-23	24,300[b]	18,600[b]	23,000[b]	24,200[b]	17,000[b]
10-24[c]	26,000[b]	22,500[b]	24,776	30,300[b]	19,900[b]
10-27[c]	26,300[b]	19,100[b]	22,487	26,300[b]	19,600[b]
10-28	12,658[b]	12,306[b]	12,953	18,500[b]	14,066[b]
10-29	5,899	6,138	4,822	7,120[b]	5,946
10-30	6,387	10,442	5,286	9,829	6,885
10-31	4,253	7,228[b]	6,905	14,127	4,936
11-1	5,507[b]	7,190[b]	5,760[b]	D	7,401
11-2	5,940[b]	7,920[b]	6,733[b]	D	9,212
11-3	5,837[b]	5,658[b]	5,543[b]	D	6,783[b]
11-4	4,013[b]	5,081[b]	5,826[b]	D	6,848[b]
11-5	3,816[b]	4,257[b]	D	D	8,227[b]
11-6	D	4,956[b]	D	D	D
11-7	D	4,411[b]	D	D	D
	Group IV HCV-11325				
	13023	13024	13143	13144	13145
11-12	13,616[b]	12,469[b]	20,700[b]	17,000[b]	13,594
11-13	20,497[b]	16,900[b]	25,600[b]	26,500[b]	20,398[b]
11-14[c]	18,500[b]	12,800[b]	21,400[b]	19,100[b]	16,000[b]
11-17[c]	16,900[b]	11,293[b]	19,300[b]	14,045[b]	13,993[b]
11-19	19,500[b]	10,837[b]	14,994[b]	12,335[b]	24,233[b]
11-21	7,966[b]	8,329[b]	7,604[b]	9,732[b]	8,380[b]
11-24	9,535[b]	5,938[b]	10,669[b]	10,076[b]	7,802[b]
11-26	5,866[b]	5,607[b]	6,370[b]	7,977[b]	4,352[b]
11-28	4,549[b]	7,002[b]	8,723[b]	6,943[b]	6,764[b]
12-1	D	4,730[b]	4,905[b]	D	5,845[b]
12-3	D	7,702[b]	2,507	D	5,002[b]
12-5	D	3,573[b]	D	D	4,889[b]
12-8	D	3,964[b]	D	D	4,963[b]
12-10	D	4,622[b]	D	D	7,455[b]
12-12	D	6,083	D	D	21,400[b]

penic range within 96 hours after infection. It should be pointed out that 4 of 5 animals in Group II had WBC counts well above leukopenic levels at various times after the initial leukopenia occurred. Significant differences occurred in the mean values of Group I on days 1 through 8; Group III on days 1 through 10; Group II on days 2 through 10 and Group IV on days 2 through 23.

Animal 12941 did not manifest either a profound leukopenia or decrease in ESR until approximately 120 hours after the other animals in Group II were showing these changes. A fluorescent antibody serum neutralization test was conducted on pre-infection serum from this animal by Dr. W. C. Stewart, of the Virology Section, Diagnostic Services, NADL, Ames, Iowa. A neutralization titer of 0.6 against HCV was obtained with the serum. This low antibody titer, according to Dr. Stewart, was sufficient to account for the delayed reaction of the pig to HCV-11325.

Reticulocyte counts

Conspicuous reduction in reticulocyte numbers in every pig, regardless of the strain of HCV infecting them, is evident from Table 14. Groups I and III (virulent strain) had average pre-infection reticulocyte levels of 1.0 and 1.4 percent respectively (Figure 9). Forty-eight hours after infection with HCV-325 these values dropped to 0.5 and 0.4 percent. Groups II and IV, infected by the less virulent HCV-11325, had pre-

Table 14. Reticulocyte counts from blood of young pigs infected by highly virulent strain (HCV-325) or strain with reduced virulence (HCV-11325) of hog cholera virus[a]

Date	Group I HCV-325 Animal numbers				
1969	12825	12826	12827	12828	12829
8-20	7(1.4%)	10(2.0%)	4(0.8%)	3(0.6%)	3(0.6%)
8-21	8(1.6%)	6(1.2%)	3(0.6%)	2(0.4%)	5(1.0%)
8-22[b]	9(1.8%)	11(2.2%)	4(0.8%)	3(0.6%)	12(2.4%)
8-25[b]	3(0.6%)	8(1.6%)	7(1.4%)	4(0.8%)	13(2.6%)
8-26	4(0.8%)	17(3.4%)	5(1.0%)	4(0.8%)	14(2.8%)
8-27	1(0.2%)	1(0.2%)	5(1.0%)	3(0.6%)	3(0.6%)
8-28	2(0.4%)	0	2(0.4%)	7(1.4%)	4(0.8%)
8-29	1(0.2%)	10(2.0%)	D[c]	3(0.6%)	4(0.8%)
8-30	0	3(0.6%)	D	D	2(0.4%)
8-31	0	D	D	D	4(0.8%)
9-1	1(0.2%)	D	D	D	1(0.2%)
9-2	0	D	D	D	0
9-3	0	D	D	D	3(0.6%)
	Group II HCV-11325				
	12941	12942	12943	12944	12945
9-10	6(1.2%)	13(2.6%)	15(3.0%)	13(2.6%)	12(2.4%)
9-11	13(2.6%)	17(3.4%)	18(3.6%)	17(3.4%)	19(3.8%)
9-12[b]	15(3.0%)	14(2.8%)	13(2.6%)	18(3.6%)	25(5.0%)
9-15[b]	18(3.6%)	20(4.0%)	13(2.6%)	9(1.8%)	16(3.6%)
9-17	7(1.4%)	9(1.8%)	6(1.2%)	8(1.6%)	13(2.6%)
9-19	1(0.2%)	5(1.0%)	4(0.8%)	4(0.8%)	13(2.6%)
9-22	6(0.4%)	1(0.2%)	0	1(0.2%)	2(0.4%)
9-24	14(2.8%)	0	0	1(0.2%)	2(0.4%)
9-26	4(0.8%)	2(0.4%)	3(0.6%)	1(0.2%)	0
9-29	0	1(0.2%)	1(0.2%)	0	3(0.6%)
10-1	2(0.4%)	0	1(0.2%)	1(0.2%)	8(1.6%)
10-3	1(0.2%)	D	0	0	4(0.8%)
10-6	5(1.0%)	D	D	3(0.6%)	10(2.0%)
10-8	1(0.2%)	D	D	1(0.2%)	13(2.6%)
10-10	0	D	D	D	8(1.6%)
10-13	0	D	D	D	10(2.0%)
10-15	0	D	D	D	13(2.6%)

[a]Reticulocytes reported in number counted/500 red blood cells with percentage in parenthesis.

[b]Date of infection.

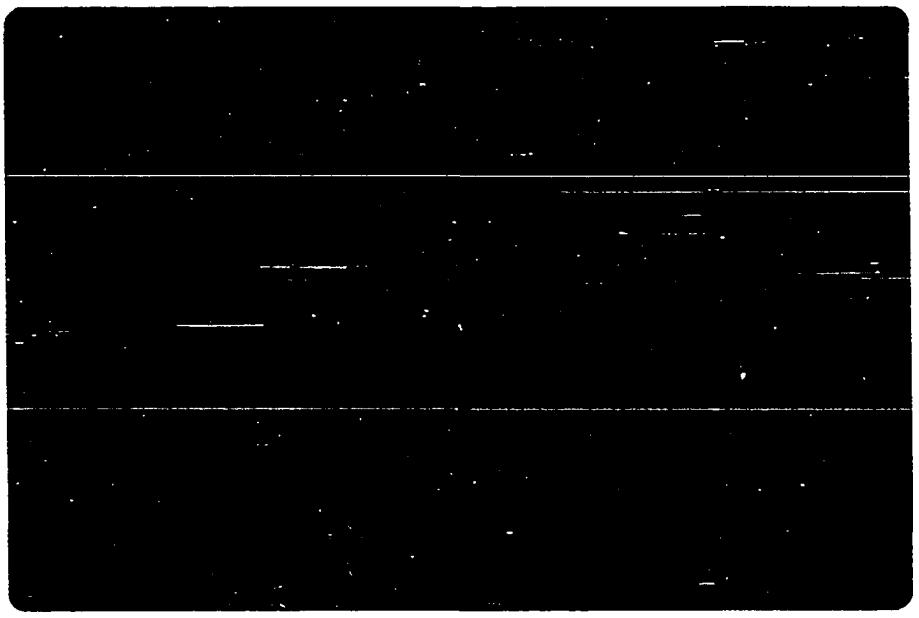
[c]D-animal died.

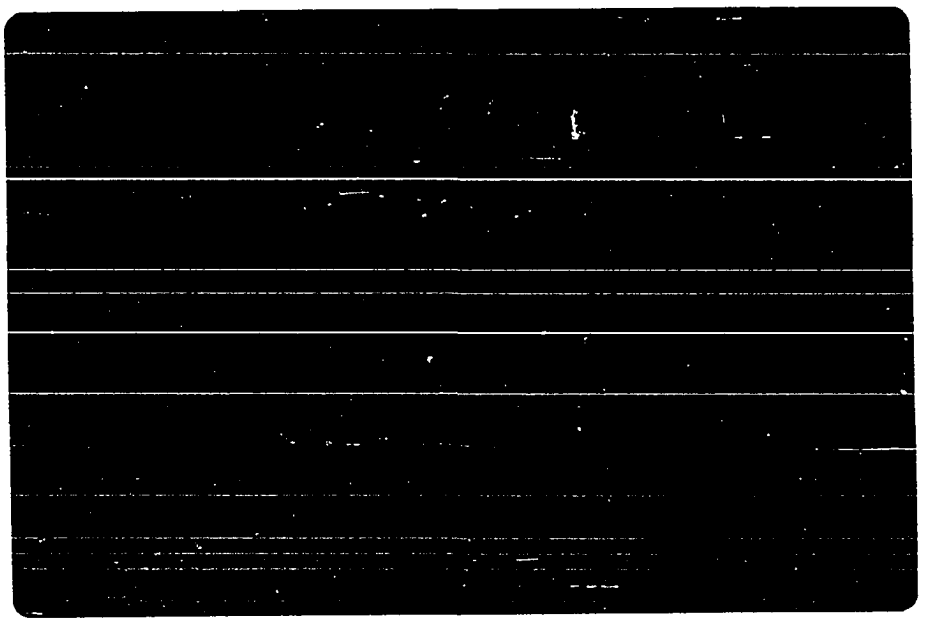
Table 14 (Continued)

Date	Group III HCV-325 Animal numbers				
1969	13136	13137	13138	13139	13140
10-22	7(1.4%)	7(1.4%)	7(1.4%)	6(1.2%)	10(2.0%)
10-23	11(2.2%)	4(0.8%)	10(2.0%)	5(1.0%)	7(1.4%)
10-24[b]	8(1.6%)	7(1.4%)	8(1.6%)	5(1.0%)	10(2.0%)
10-27[b]	8(1.6%)	5(1.0%)	10(2.0%)	4(0.8%)	5(1.0%)
10-28	3(0.6%)	4(0.8%)	2(0.4%)	5(1.0%)	4(0.8%)
10-29	2(0.4%)	2(0.4%)	4(0.8%)	2(0.4%)	1(0.2%)
10-30	1(0.2%)	4(0.8%)	4(0.8%)	6(1.2%)	4(0.8%)
10-31	1(0.2%)	1(0.2%)	2(0.4%)	2(0.4%)	4(0.8%)
11-1	2(0.4%)	4(0.8%)	2(0.4%)	D	1(0.2%)
11-2	3(0.6%)	4(0.8%)	3(0.6%)	D	4(0.8%)
11-3	3(0.6%)	1(0.2%)	1(0.2%)	D	1(0.2%)
11-4	2(0.4%)	4(0.8%)	3(0.6%)	D	0
11-5	1(0.2%)	1(0.2%)	D	D	1(0.2%)
11-6	1(0.2%)	D	D	D	D
11-7	0	D	D	D	D
	Group IV HCV-11325				
	13023	13024	13143	13144	13145
11-12	13(2.6%)	20(4.0%)	16(3.2%)	12(2.4%)	17(3.4%)
11-13	13(2.6%)	16(3.2%)	15(3.0%)	17(3.4%)	16(3.2%)
11-14[b]	7(1.4%)	16(3.2%)	11(2.2%)	15(3.0%)	10(2.0%)
11-17[b]	3(0.6%)	17(3.4%)	8(1.6%)	15(3.0%)	6(1.2%)
11-19	3(0.6%)	15(3.0%)	6(1.2%)	8(1.6%)	7(1.4%)
11-21	1(0.2%)	5(1.0%)	1(0.2%)	0	1(0.2%)
11-24	1(0.2%)	0	1(0.2%)	1(0.2%)	0
11-26	0	1(0.2%)	0	0	0
11-28	0	2(0.4%)	0	0	0
12-1	D	0	1(0.2%)	D	0
12-3	D	1(0.2%)	33(6.6%)	D	0
12-5	D	1(0.2%)	D	D	1(0.2%)
12-8	D	3(0.6%)	D	D	0
12-10	D	5(1.0%)	D	D	1(0.2%)
12-12	D	9(1.8%)	D	D	2(0.4%)

Figure 9. Reticulocyte in smear of peripheral blood collected prior to infection. Brilliant cresyl blue counterstained with Wright's stain. 1000X

Figure 10. Peripheral blood with Howell-Jolly body in upper left corner and nucleated red blood cell in lower right. Wright's stain. 1000X





infection reticulocyte levels of 2.5 and 2.1 percent. Forty-eight hours p.i. they had levels of 1.7 and 1.6 percent. These levels continued dropping until, after 120 hours, the reticulocyte levels were 0.40 and 0.12 percent respectively. Most of the animals which survived the period of the study had abnormally low reticulocyte levels. A few animals developed unusually large numbers of reticulocytes following the initial reduction.

Howell-Jolly bodies

Rarely were Howell-Jolly (H-J) bodies (Figure 10) observed in all 5 animals of a group on a single day. The small number of H-J bodies present did not permit an accurate assessment of changes which may have occurred as a result of infection by HCV.

Differential leukocyte counts

The number of lymphocytes and polymorphonuclear neutrophilic leukocytes (PMN) in pre-infection blood smears varied a great deal between samples taken from the same animal on consecutive days, and between samples taken from different animals of the same group on the same day. A much larger number of immature WBC forms were observed than was expected on the basis of published reports. Neutrophilic metamyelocytes were the most frequently observed of the immature forms.

Lymphocytes were the predominant WBC's observed in blood smears collected before infection from 3 of the 5 pigs in

Group I (highly virulent). Segmented PMN leukocytes were predominant in the smears from the other 2 animals. Within 24 hours after infection by HCV-325, a severe leukopenia developed. In 4 of the 5 animals in Group I, the most severe reduction was in the number of lymphocytes. In 3 of the 4 animals, the number of lymphocytes was reduced to one-half their pre-infection level and in the fourth animal, the lymphocytes were reduced to one-fourth of their pre-infection level. In the fifth animal, the most severe reduction was in the PMN leukocytes, which were reduced to less than one-half of their pre-infection level. Following the initial severe drop in numbers, neither lymphocytes nor PMN leukocytes were again detected at their pre-infection levels. A general increase in immature PMN leukocytes, particularly neutrophilic bands and metamyelocytes, occurred at the same time that an eosinopenia was occurring. Monocyte numbers did not vary remarkably during the course of the disease.

Effects of infection by HCV-11325 (less virulent) in the 5 animals of Group II were much less severe and more prolonged in onset than those described in Group I. Lymphocytes were the prevalent WBC's in pre-infection blood smears from 2 animals and PMN leukocytes in 2 others, while the fifth animal's blood smears varied daily as to which of the 2 cell types was most commonly observed. The typical hematologic response to HCV infection was not observed in animal 12941 because of its immune status discussed previously. In the other four animals,

lymphopenia developed approximately 96 hours after infection. Neutropenia developed somewhat later in three of the animals; however, animal 12945 did have a PMN count of 4890 within 96 hours after infection. Although quite variable between animals and between day to day blood samples, an increase in immature PMN leukocytes, a decrease in eosinophils and no change in the numbers of monocytes was also observed.

Four of the five pigs in Group III (virulent) had a majority of lymphocytes, while in the fifth animal, PMN leukocytes were the most frequently encountered cell in smears of blood collected prior to infection. Following inoculation of HCV-325 into the animals, a sequence of events which were a repetition of those experienced by the Group I animals occurred. Within 24 hours a precipitous leukopenia occurred. The major part of the cell losses were due to the severe lymphopenia which occurred in all five animals. During the same period, a modest increase in PMN leukocytes was observed in 4 of the animals. Increased numbers of immature PMN leukocytes, reduced numbers of eosinophilic leukocytes and monocyte numbers which were not significantly affected by the HCV were also observed in blood smears from the animals in Group III.

Blood smears collected prior to infection from 3 of the 5 animals in Group IV (less virulent) contained more PMN leukocytes than lymphocytes. The opposite situation prevailed with the other 2 animals. Even before the leukopenia following inoculation of HCV-11325 into the animals was obvious, a mild

reduction of lymphocytes was observed. This occurred within 48 hours after infection. During the same period a transient and modest elevation of PMN leukocytes was recorded. After 48 hours of infection both cell types were greatly reduced in number, a condition which persisted until the animal died or the experiment was terminated.

Most responses of the blood and blood-forming organs of the infected pigs were basically similar irregardless with which strain they were infected. Two quantitative differences were reflected by the infected animals. Most apparent was the difference in time between inoculation of the virus and the appearance of the leukopenia. A less obvious difference was the ability of the animals hemopoietic system to restore TWBC levels after the initial leukopenia. The devastating action of the highly virulent HCV-325 produced a quick reduction of leukocyte numbers and apparently inflicted a severe injury to the bone marrow from which the animal rarely, if ever, recovered.

Nucleated red blood cells

Blood smears collected from the 20 animals prior to infection with either strain of HCV rarely contained one nucleated erythrocyte and never more than two nucleated RBC's per 100 leukocytes counted. An increase in numbers of nucleated RBC's did occur at varying periods after infection (Figure 10). In animals of Group I (virulent) the most consistent

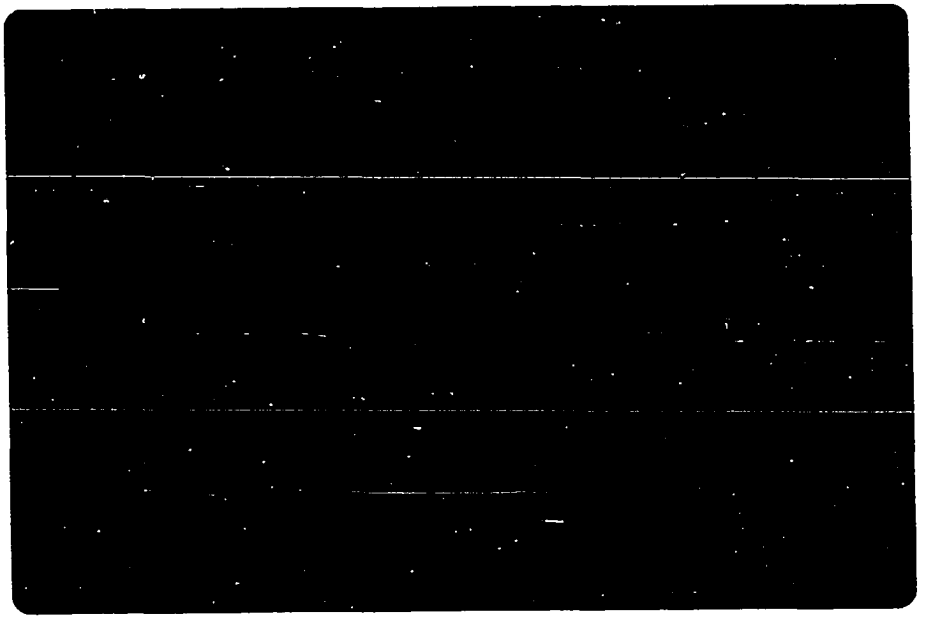
increase occurred at 72 hours after inoculation of the virus. From 3 to 45 of the immature red cells, per 100 leukocytes counted, were present in the blood of animals showing the increase. Blood from animals in Group II (less virulent) did not show a significant increase in these cells. An increase in nucleated RBC's was also observed in the animals of Group III (virulent) which were infected by the virulent HCV-325. Blood from these animals, as that from the animals in Group I, showed the most marked increase in immature RBC's around 72 hours after infection. The increases in Group III animals ranged from 3 to 70 nucleated RBC's per 100 leukocytes. Smears of blood from animals in Group IV (less virulent), as that from animals of Group II, did not show a significant increase. These findings suggest that the increase in numbers of immature RBC's following infection may be related to the virulence of the virus.

Bone marrow smears

Asynchronous development of the bone marrow cells was occasionally observed in samples collected prior to, and after infection. Most often the cell would resemble a band or metamyelocyte PMN or eosinophilic leukocyte, but would have an homogeneous basophilic cytoplasm. No unusual forms of cells were observed in bone marrow of infected animals, which were not present in equal numbers in bone marrow cells of normal animals (Figures 11 through 14). The most significant obser-

Figure 11. Bone marrow smear with promyelocyte undergoing mitosis and 2 polymorphonuclear neutrophilic leukocytes. Wright's stain. 1000X

Figure 12. Bone marrow smear containing a segmented polymorphonuclear neutrophilic leukocyte, blood platelets, polychromatophilic rubricyte, metarubricyte, and a band polymorphonuclear neutrophilic leukocyte. Wright's stain. 1000X



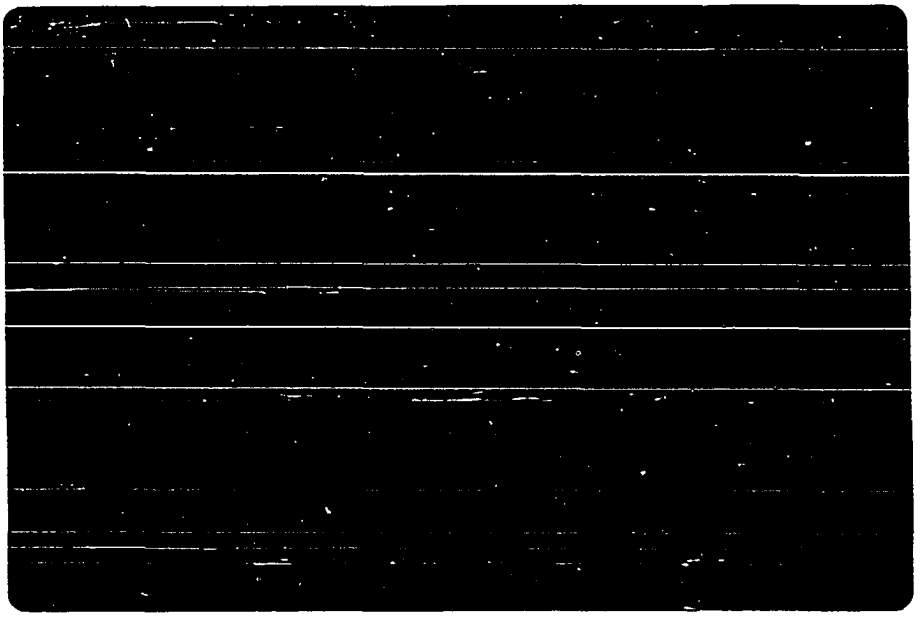
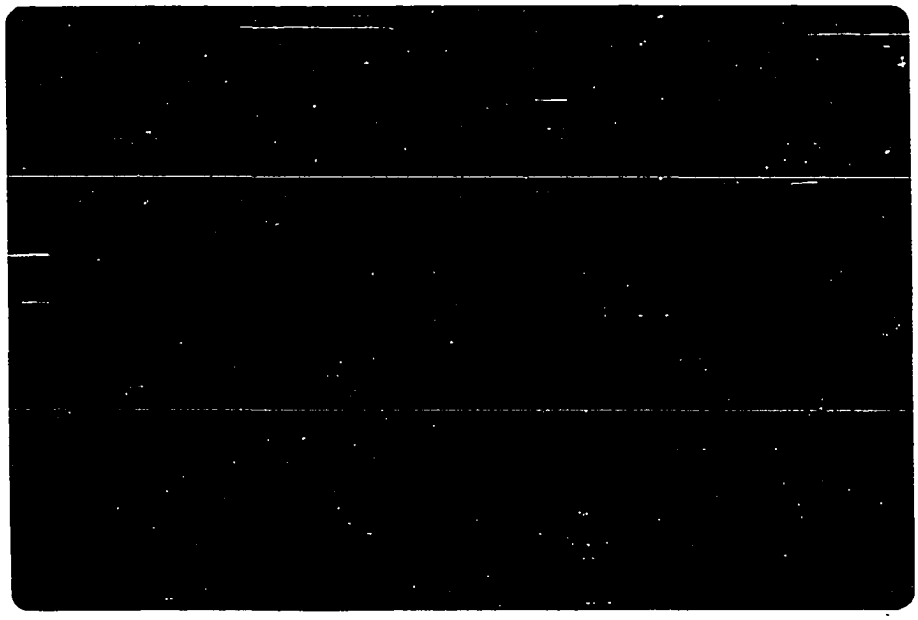
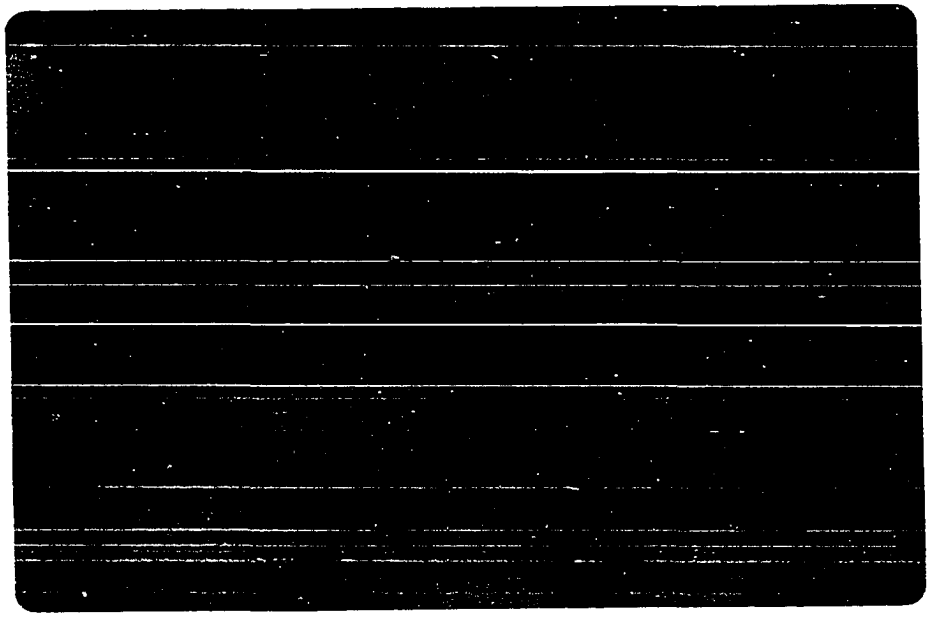


Figure 13. Bone marrow smear containing 2 prorubricytes, a rubricyte and segmented polymorphonuclear neutrophilic leukocyte. Wright's stain. 1000X

Figure 14. Bone marrow smear with eosinophilic metamyelocyte, neutrophilic myelocyte, neutrophilic metamyelocyte, band neutrophil, segmented neutrophil and one unidentified cell. Wright's stain. 1000X





vation in regard to cell morphology was the presence of large numbers of dead cells present in bone marrow collected from HCV infected animals that were in the leukopenic phase. The cells, of various sizes, consisted primarily of homogeneous, lavender-colored cytoplasm and were in various stages of structural dissolution. On low power microscopic examination, the large number of these cellular remnants and paucity of normal bone marrow cellular elements was obvious (Figure 15). Megakaryocytes were rarely observed and those in the infected animals were generally degenerated. This type of examination is not suitable for quantitation of cells of any type.

Examination of Table 15 shows the lack of any consistent relationship between the ratio of myeloid to erythroid bone marrow cells and the TWBC number. The myeloid:erythroid ratio (M:E) describes the relationship of all granulocytic cells of the bone marrow to nucleated red blood cells. It is obtained by dividing the total number of nucleated erythrocytic cells into the sum of all the granulocytic cells. In all four groups of animals the M:E became elevated at varying periods of time after infection. Some of these increases were extremely high, e.g., 37.66, 27.50. Total WBC's in these animals were in the leukopenic range at the time and remained quite low.

Serum Protein Studies

Total serum proteins

All animals in Groups I and III (virulent) had blood serum protein levels which either remained the same as before infec-

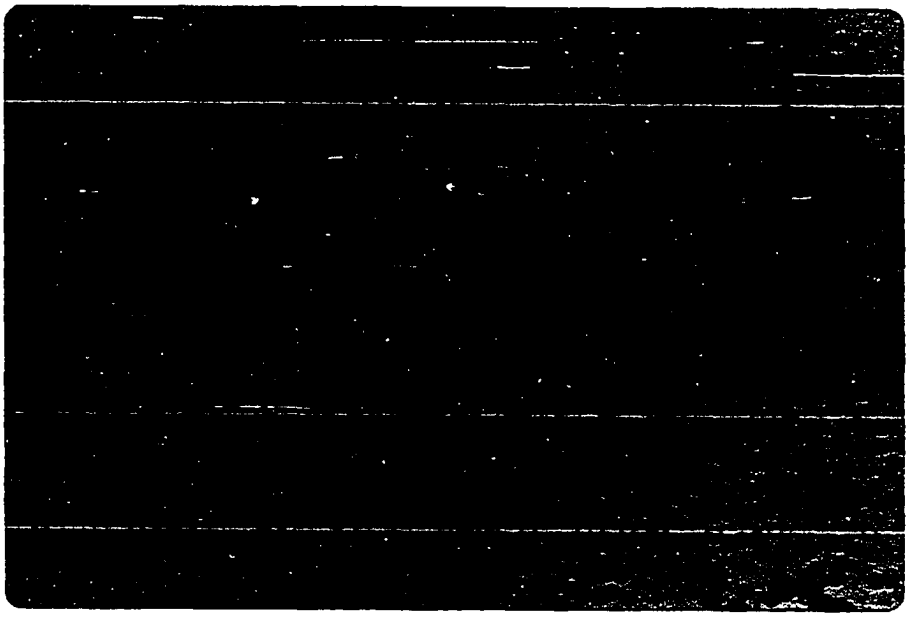


Figure 15. Bone marrow smear containing large numbers of degenerating cells. Material was collected while animal's total white blood cell count was in the leukopenic range. Wright's stain. 250X

Table 15. Bone marrow observations from young pigs infected with a highly virulent (HCV-325) or moderately virulent (HCV-11325) hog cholera virus

Group I HCV-325									
	Dates	8-20	8-21	8-22	8-25[a]	8-26	8-27	8-28	8-29
Animal numbers									
12825	M:E[b]	1.37	1.81	1.91	1.72	0.98	0.45	0.29	0.20
	TWBC[c]	21,582	32,836	28,557	24,158	13,731	8,684	2,915	4,436
12826	M:E	0.62	0.85	1.05	0.45	0.92	1.21	1.16	2.10
	TWBC	19,303	23,500	30,149	19,300	9,106	5,461	6,739	7,349
12827	M:E	2.38	3.45	4.36	1.43	1.63	2.65	1.67	
	TWBC	18,408	17,800	20,600	17,900	13,069	6,714	6,102	
12828	M:E	1.78	2.79	1.68	2.78	2.15	2.00	3.47	3.77
	TWBC	20,500	19,800	19,800	20,900	12,000	5,369	7,429	8,143
12829	M:E	1.85	2.41	1.45	1.89	1.79	1.20	1.33	0.96
	TWBC	21,700	21,194	23,000	24,158	8,816	4,577	5,181	3,836
Group II HCV-11325									
	Dates	9-10	9-11	9-12	9-15[a]	9-17	9-19	9-22	9-24
12941	M:E	2.61	3.0	1.29	2.30	7.43	3.50	2.77	2.58
	TWBC	24,975	25,100	25,300	25,600	31,243	32,100	29,200	25,100
12942	M:E	1.40	1.72	3.50	1.75	5.26	2.82	5.14	7.18
	TWBC	28,500	18,507	21,000	21,000	15,522	12,935	13,930	7,227
12943	M:E	1.49	1.06	0.59	1.32	2.53	1.92	2.97	14.86
	TWBC	27,064	26,700	25,000	26,100	17,910	16,500	12,600	7,590
12944	M:E	1.07	1.45	1.65	2.94	4.77	3.63	9.78	8.84
	TWBC	18,905	18,800	19,400	34,500	20,800	18,831	11,244	10,800
12945	M:E	2.99	1.39	2.48	2.46	5.21	1.50	1.23	11.10
	TWBC	34,726	35,800	31,400	25,900	25,100	12,700	8,663	9,603

[a]Date of infection.

[b]Myeloid:Erythroid ratio.

[c]TWBC reported as number of cells/cu. mm.

8-30	8-31	9-1	9-2	9-3
5.83	5.85	13.33	11.58	3.66
7,929	6,600	5,670	6,190	5,237
5.23				
8,522				
2.35	5.83	10.88	8.29	6.00
5,403	7,222	7,208	7,390	5,171

9-26	9-29	10-1	10-3	10-6	10-8	10-10	10-13	10-15
1.04	4.80	3.20	15.12	3.27	5.52	3.83	3.92	2.98
14,900	11,916	7,638	11,048	13,513	9,349	5,958	6,336	25,600
26.55	7.74	3.98						
15,100	8,500	11,075						
4.74	4.47	5.05	6.31					
8,375	8,213	10,141	13,272					
5.79	15.62	4.47	2.86	2.90	1.61			
10,400	7,790	5,907	6,465	6,299	3,537			
4.33	1.86	0.66	3.84	4.43	2.19	3.77	7.78	8.0
7,590	5,993	8,396	10,869	14,203	21,293	24,477	27,300	25,900

Table 15. (Continued)

Group III HCV-325									
	Dates	10-22	10-23	10-24	10-27[a]	10-28	10-29	10-30	10-31
Animal numbers									
13136	M:E	1.98	2.77	2.20	1.46	3.15	1.50	7.23	2.73
	TWBC	25,700	24,300	26,000	26,300	12,658	5,899	6,387	4,253
13137	M:E	2.73	2.42	3.64	3.03	2.46	2.68	2.50	4.72
	TWBC	22,200	18,600	22,500	19,100	12,306	6,138	10,442	7,228
13138	M:E	1.49	1.65	1.20	1.38	0.75	0.83	1.89	1.03
	TWBC	25,400	23,000	24,776	22,487	12,953	4,822	6,286	6,905
13139	M:E	4.56	5.53	2.60	2.50	2.12	2.10	0.77	1.48
	TWBC	26,800	24,200	30,300	18,500	18,500	7,120	9,829	14,127
13140	M:E	3.41	2.66	3.12	1.87	3.47	1.09	0.65	1.55
	TWBC	20,500	17,800	19,900	14,066	14,066	5,946	6,885	4,936
Group IV HCV-11325									
	Dates	11-12	11-13	11-14	11-17[a]	11-19	11-21	11-24	11-26
13023	M:E	1.62	3.01	3.77	7.0	3.27	1.30	7.84	1.19
	TWBC	13,616	20,497	18,500	16,900	19,500	7,966	9,535	5,866
13024	M:E	0.54	1.34	1.29	2.07	5.91	0.91	3.05	1.56
	TWBC	12,469	16,900	12,800	11,293	10,837	8,329	5,938	5,607
13143	M:E	2.24	2.13	3.75	3.17	2.86	6.46	23.8	18.83
	TWBC	20,700	25,600	21,400	19,300	14,994	7,604	10,669	6,370
13144	M:E	1.25	1.35	2.11	3.16	7.26	4.10	7.23	10.32
	TWBC	17,000	26,546	19,100	14,045	12,335	9,732	10,076	7,977
13145	M:E	1.44	1.24	2.34	2.53	9.38	1.72	4.67	18.57
	TWBC	13,594	20,398	16,000	13,993	24,233	8,380	7,802	4,352

11-1	11-2	11-3	11-4	11-5	11-6	11-7
7.68	7.83	17.71	10.72	2.37		
5,507	5,940	5,837	4,013	3,816		
4.71	9.92	22.33	7.34	3.32	2.07	1.52
7,190	7,920	5,658	5,081	4,257	4,956	4,411
4.06	13.0	37.66	7.61			
5,760	6,733	5,543	5,826			
0.64	18.71	14.06	6.14	4.11		
7,401	9,212	6,783	6,848	8,227		

11-28	12-1	12-3	12-5	12-8	12-10	12-12
2.11						
4,549						
13.56	18.08	5.73	6.87	2.04	1.17	0.92
7,002	4,730	7,702	3,573	3,964	4,622	6,083
27.50	4.15	0.76				
8,723	4,905	2,507				
26.2						
6,943						
26.10	12.45	7.15	4.74	1.70	1.10	2.53
6,764	5,845	5,002	4,889	4,963	7,455	21,400

tion or showed a slight increase (Table 16). Serum proteins of all animals in Group II, and 4 of the 5 in Group IV, infected by the moderately virulent HCV-11325 decreased slightly during the course of the disease. There was a marked lack of consistency between day to day levels of serum proteins in the individual animals during the course of the experimentally-induced HC. The majority of the changes were so minor that they could not be considered as a reliable indicator of change.

Electrophoretic separation of serum proteins

Ninety-five percent of the animals infected with the 2 hog cholera viruses experienced a minor reduction in blood serum albumin and an apparent increase in the total serum globulins (Table 17). A major part of this consistent elevation was apparently due to the alpha globulins. Ninety percent of the pigs infected with the more virulent HCV-325 experienced some slight increase of alpha globulin during HC infection. Of the animals infected with the strain of lesser virulence (HCV-11325), 80 percent had moderately elevated alpha globulin fractions. An opposite situation prevailed in the case of the gamma fraction of the blood serum globulins. The highly virulent HCV caused some degree of reduction below pre-infection levels in 60 percent of the animals infected by it. In contrast the less virulent HCV produced a similar decrease in the immune globulin-bearing-fraction of the blood serum proteins in only 30 percent of its hosts. It should be stressed that these changes in levels of serum protein

Table 16. Effect of infection by highly virulent (HCV-325) or moderately virulent (HCV-11325) hog cholera viruses on the total protein content of blood serum in young pigs[a]

Date	Group I HCV-325 Animal numbers				
1969	12825	12826	12827	12828	12829
8-20	5.5	5.1	6.5	5.3	5.4
8-21	5.0	4.8	6.0	5.0	5.6
8-22[b]	5.8	5.4	6.6	6.4	5.6
8-25[b]	6.3	6.4	6.3	6.2	6.4
8-26	6.3	6.4	6.3	6.3	6.4
8-27	6.0	6.4	7.0	6.6	6.4
8-28	6.2	6.4	6.8	6.7	6.0
8-29	6.2	6.3	D[c]	7.5	6.5
8-30	6.4	6.0	D	D	6.6
8-31	5.9	D	D	D	6.0
9-1	6.0	D	D	D	6.2
9-2	5.9	D	D	D	6.0
9-3	5.9	D	D	D	5.9
	Group II HCV-11325				
	12941	12942	12943	12944	12945
9-10	6.4	6.6	6.4	6.4	6.2
9-11	6.5	6.3	6.3	6.2	6.1
9-12[b]	6.4	6.1	6.5	6.3	6.2
9-15[b]	6.2	6.1	6.4	6.1	6.3
9-17	6.8	6.0	6.4	6.0	6.1
9-19	6.0	5.9	5.8	5.7	5.8
9-22	6.0	5.8	5.8	5.8	5.8
9-24	6.0	6.0	6.0	6.0	6.0
9-26	6.0	6.0	5.9	5.9	5.9
9-29	5.8	5.9	5.9	6.0	6.0
10-1	6.0	5.9	5.8	5.6	6.0
10-3	6.0	D	5.9	5.8	5.9
10-6	5.9	D	D	5.8	6.0
10-8	5.8	D	D	5.8	5.9
10-10	5.8	D	D	D	5.9
10-13	5.8	D	D	D	5.9
10-15	5.6	D	D	D	5.9

[a]Total protein reported in gm./100 ml.

[b]Date of infection.

[c]D-animal died.

Table 16 (Continued)

Date	Group III HCV-325 Animal numbers				
1969	13136	13137	13138	13139	13140
10-22	6.0	6.1	6.2	6.0	6.0
10-23	6.1	6.1	6.1	6.1	6.1
10-24[b]	6.1	6.2	6.1	6.1	6.1
10-27[b]	6.0	6.1	6.1	6.0	6.0
10-28	6.2	6.2	6.2	6.2	5.9
10-29	6.0	6.2	6.2	6.0	6.0
10-30	6.0	6.1	6.1	6.1	6.1
10-31	6.0	6.0	6.0	6.3	6.2
11-1	6.0	6.1	6.0	D	6.1
11-2	6.0	6.1	6.1	D	6.0
11-3	6.1	6.0	6.1	D	6.1
11-4	6.0	6.1	6.2	D	6.1
11-5	6.1	6.0	D	D	6.0
11-6	D	6.0	D	D	D
11-7	D	6.4	D	D	D
	Group IV HCV-11325				
	13023	13024	13143	13144	13145
11-12	6.2	6.1	6.0	6.0	6.1
11-13	6.1	6.1	6.1	6.1	6.1
11-14[b]	6.0	6.0	6.1	6.0	6.0
11-17[b]	6.0	6.0	6.0	6.0	6.0
11-19	6.1	6.1	6.1	6.0	6.0
11-21	6.1	6.1	6.1	6.1	6.1
11-24	6.4	6.0	6.0	5.9	6.0
11-26	6.2	6.0	6.0	6.0	6.0
11-28	5.8	6.1	6.0	5.5	6.0
12-1	D	6.1	4.7	D	6.0
12-3	D	6.2	4.4	D	5.5
12-5	D	5.6	D	D	5.3
12-8	D	5.6	D	D	5.7
12-10	D	6.2	D	D	6.2
12-12	D	6.6	D	D	6.0

Table 17. The effect of infection by hog cholera viruses of high (HCV-325) and moderate virulence (HCV-11325) on the serum protein fractions of young pigs[a]

Pig numbers	Group I Dates												
	8-20	8-21	8-22	8-25[b]	8-26	8-27	8-28	8-29	8-30	8-31	9-1	9-2	9-3
12825													
Alb.	47.4	44.1	41.5	46.8	43.8	41.6	39.6	38.3	34.9	34.6	34.8	35.0	34.4
α	26.0	26.5	27.7	25.7	27.5	27.2	30.3	30.4	35.4	34.6	35.2	34.2	34.4
B	16.2	10.0	17.0	17.4	19.3	17.6	16.1	19.1	19.7	20.5	16.0	16.2	18.0
γ	10.4	11.4	13.8	10.1	9.4	13.6	14.1	12.2	10.0	10.3	14.0	14.5	13.2
Glob.	52.6	55.9	58.5	53.2	56.2	58.4	60.4	61.7	65.1	65.4	65.2	65.0	65.6
12826													
Alb.	47.7	45.2	45.1	45.6	41.5	42.9	41.6	43.2	36.6	D[c]	D	D	D
α	25.3	27.4	27.7	29.2	24.5	30.0	30.8	25.9	36.2	D	D	D	D
B	16.1	17.0	16.9	17.9	20.2	16.2	16.3	19.5	18.1	D	D	D	D
γ	10.9	10.4	10.3	7.2	13.8	10.9	11.3	11.4	9.1	D	D	D	D
Glob.	52.3	54.8	54.9	54.4	58.5	57.1	58.4	56.8	63.4	D	D	D	D
12827													
Alb.	38.0	40.5	41.6	44.1	45.0	42.0	39.9	D	D	D	D	D	D
α	26.7	25.4	24.9	25.4	31.6	24.7	28.0	D	D	D	D	D	D
B	23.9	21.4	21.1	15.8	14.8	20.0	21.5	D	D	D	D	D	D
γ	11.4	12.7	12.4	14.7	8.6	13.3	10.6	D	D	D	D	D	D
Glob.	62.0	59.5	58.4	55.9	55.0	58.0	60.1	D	D	D	D	D	D

12828													
Alb.	44.6	40.1	43.6	42.9	43.8	42.0	43.0	41.5	D	D	D	D	D
α	32.2	33.8	31.9	32.1	27.4	32.4	32.4	32.1	D	D	D	D	D
B	15.1	15.2	14.7	15.8	17.8	15.0	14.0	15.6	D	D	D	D	D
γ	8.1	10.9	9.8	9.2	11.0	10.6	10.6	10.8	D	D	D	D	D
Glob.	55.4	59.9	56.4	57.1	56.2	58.0	57.0	58.5	D	D	D	D	D
12829													
Alb.	i.s.[d]	44.4	44.7	46.3	45.1	46.7	41.4	38.9	37.2	38.9	37.1	38.2	41.5
α	i.s.	27.0	27.7	25.6	29.0	26.7	29.0	30.8	33.0	30.1	31.4	31.2	30.9
B	i.s.	15.7	17.0	18.2	17.2	14.8	16.7	19.2	20.1	16.8	18.1	16.8	17.9
γ	i.s.	13.0	10.6	10.0	8.8	11.9	13.0	11.1	9.7	14.2	13.3	13.8	9.7
Glob.	i.s.	55.6	55.3	53.7	54.9	53.3	58.6	61.1	62.8	61.1	62.9	61.8	58.5

[a]Protein fractions reported in percentage.

[b]Date of infection.

[c]D-animal died.

[d]i.s.-Insufficient sample.

Table 17. (Continued)

Pig numbers	Group II Dates															
	9-10	9-11	9-12	9-15[b]	9-17	9-19	9-22	9-24	9-26	9-29	10-1	10-3	10-6	10-8	10-10	10-13	10-15
12941																	
Alb.	39.7	38.1	38.0	39.3	38.5	36.6	36.3	34.7	34.8	31.0	29.6	30.0	29.9	29.1	27.4	24.2	23.6
α	29.6	29.5	30.1	28.5	30.1	30.3	28.4	30.5	31.0	35.7	38.3	35.2	28.2	26.5	28.7	30.0	26.9
B	13.2	14.2	14.1	15.3	15.0	17.5	19.9	16.0	14.3	14.7	18.1	15.0	15.6	15.7	16.8	19.1	21.1
γ	17.5	18.2	17.8	16.9	16.4	15.6	15.4	18.7	19.9	18.6	14.1	19.8	26.3	28.8	27.1	26.7	28.4
Glob.	60.3	61.9	62.0	60.7	61.5	63.4	63.7	65.3	65.2	69.0	70.4	70.0	70.1	70.9	72.6	75.8	76.4
12942																	
Alb.	43.6	40.4	39.3	41.7	41.4	38.7	35.3	34.2	35.0	34.9	32.6	D	D	D	D	D	D
α	27.5	29.6	29.5	27.5	27.6	29.7	32.9	34.7	34.5	32.7	35.7	D	D	D	D	D	D
B	12.9	14.6	13.5	13.9	14.3	14.5	15.6	15.7	15.0	15.4	13.3	D	D	D	D	D	D
γ	16.0	15.4	17.8	16.9	16.7	17.1	16.3	15.4	15.5	17.0	18.4	D	D	D	D	D	D
Glob.	56.4	59.6	60.7	58.3	58.6	61.3	64.7	65.8	65.0	65.1	67.4	D	D	D	D	D	D
12943																	
Alb.	35.9	34.4	34.2	36.2	36.6	34.0	31.7	31.3	34.2	31.1	29.1	28.9	D	D	D	D	D
α	26.5	30.0	29.5	26.5	23.4	25.4	29.9	31.9	29.7	25.4	29.4	28.2	D	D	D	D	D
B	17.4	16.6	13.2	16.2	18.4	17.3	16.9	19.6	15.5	21.3	16.7	16.6	D	D	D	D	D
γ	20.2	19.0	23.2	21.0	21.6	23.3	21.6	17.2	20.6	22.2	24.8	26.4	D	D	D	D	D
Glob.	64.1	65.6	65.8	63.8	63.4	66.0	68.3	68.7	65.8	68.9	70.9	71.1	D	D	D	D	D

12944

Alb.	40.3	42.4	37.8	40.1	40.7	37.3	i.s.	34.1	36.1	31.9	28.6	26.3	24.7	23.3	D	D	D
α	27.7	26.5	26.6	26.6	25.7	27.4	i.s.	29.7	26.2	23.7	24.0	25.3	27.1	31.5	D	D	D
B	15.0	12.9	16.5	15.9	16.7	17.2	i.s.	16.0	16.9	17.3	19.1	21.7	19.9	23.6	D	D	D
γ	17.0	18.2	19.1	17.4	16.9	18.2	i.s.	20.1	20.9	27.1	28.3	26.7	28.4	21.6	D	D	D
Glob.	59.7	57.6	62.2	59.9	59.3	62.7	i.s.	65.9	63.9	68.1	71.4	73.7	75.3	76.7	D	D	D

12945

Alb.	40.2	40.0	39.6	40.9	39.3	36.9	34.9	33.8	34.1	i.s.	29.5	32.0	29.1	29.4	28.8	29.3	28.8
α	29.3	30.0	29.0	26.8	25.4	27.9	28.5	32.8	33.4	i.s.	33.5	30.7	29.4	30.4	30.0	29.3	27.5
B	12.9	13.1	13.4	12.2	15.0	14.9	16.5	15.2	14.5	i.s.	14.9	13.7	14.2	15.9	14.8	14.1	14.4
γ	17.7	16.9	18.1	20.1	20.2	20.4	20.1	18.1	18.0	i.s.	22.2	23.7	27.2	24.3	26.4	27.4	29.3
Glob.	59.8	60.0	60.4	59.1	60.7	63.1	65.1	66.2	65.9	i.s.	70.5	68.0	70.9	70.6	71.2	70.7	71.2

Table 17. (Continued)

Pig numbers	Group III Dates														
	10-22	10-23	10-24	10-27[b]	10-28	10-29	10-30	10-31	11-1	11-2	11-3	11-4	11-5	11-6	11-7
13136															
Alb.	41.1	41.2	39.9	35.2	40.2	37.2	39.0	37.1	35.1	35.2	35.0	34.1	34.7	D	D
α	22.8	30.9	31.6	34.3	32.3	32.8	33.2	29.9	32.7	32.6	33.5	34.6	35.2	D	D
B	22.0	13.2	13.7	10.2	12.9	12.2	14.4	19.2	20.3	19.4	20.5	17.9	17.9	D	D
γ	14.1	14.7	14.8	20.4	14.6	17.8	13.4	13.8	11.9	12.8	11.0	13.3	12.2	D	D
Glob.	58.9	58.8	60.1	64.8	59.8	62.8	61.0	62.9	64.9	64.8	65.0	65.9	65.3	D	D
13137															
Alb.	41.1	40.9	41.1	38.9	38.1	38.4	38.5	35.0	33.7	33.1	32.5	32.8	32.9	31.9	31.8
α	27.4	29.7	30.7	31.8	29.4	30.1	30.7	27.6	30.4	31.2	31.7	32.5	31.7	32.9	33.3
B	15.5	12.8	10.8	13.7	15.0	14.3	14.1	20.3	20.0	21.3	22.8	21.8	18.3	17.3	17.1
γ	16.0	16.6	17.4	15.6	17.6	17.2	16.6	17.1	16.0	14.5	13.0	12.9	17.1	17.8	17.9
Glob.	58.9	59.1	58.9	61.1	61.9	61.6	61.5	65.0	66.3	66.9	67.5	67.2	67.1	68.1	68.2
13138															
Alb.	36.3	36.7	36.2	38.3	34.9	36.5	37.1	33.7	32.6	33.7	33.7	33.9	D	D	D
α	30.9	31.8	33.4	30.9	32.4	32.5	32.0	30.7	32.9	33.2	31.2	39.2	D	D	D
B	13.9	11.5	12.0	12.4	14.0	14.7	16.0	18.7	18.8	19.1	23.2	15.3	D	D	D
γ	18.8	20.0	18.4	18.4	18.7	16.3	14.9	16.9	15.7	14.1	11.9	11.6	D	D	D
Glob.	63.7	63.3	63.8	61.7	65.1	63.5	62.9	66.3	67.4	66.3	66.3	66.1	D	D	D

13139															
Alb.	38.8	38.3	38.3	38.7	37.1	38.7	36.8	34.3	D	D	D	D	D	D	D
α	23.7	32.2	31.4	32.6	30.3	31.9	33.8	30.5	D	D	D	D	D	D	D
B	21.7	12.1	12.6	12.5	13.9	14.8	11.5	19.5	D	D	D	D	D	D	D
γ	15.8	17.4	17.7	16.2	18.7	14.5	17.9	15.7	D	D	D	D	D	D	D
Glob.	61.2	61.7	61.7	61.3	62.9	61.3	63.2	65.7	D	D	D	D	D	D	D
13140															
Alb.	41.0	38.6	37.2	38.5	37.4	37.9	37.0	34.6	33.7	33.8	33.4	34.7	34.2	D	D
α	27.1	28.7	30.5	30.1	29.0	29.7	26.7	29.0	32.1	31.8	32.2	33.2	33.0	D	D
B	13.6	13.2	13.3	11.6	11.6	13.6	17.3	17.6	17.9	19.6	20.3	18.3	16.5	D	D
γ	18.3	19.5	19.0	19.7	22.0	18.9	19.1	18.8	16.3	14.8	14.1	13.8	16.2	D	D
Glob.	59.0	61.4	62.8	61.5	62.6	62.1	63.0	65.4	66.3	66.2	66.6	65.3	65.8	D	D

Table 17. (Continued)

Pig numbers	Group IV Dates														
	11-12	11-13	11-14	11-17[b]	11-19	11-21	11-24	11-26	11-28	12-1	12-3	12-5	12-8	12-10	12-12
13023															
Alb.	38.3	38.3	38.2	36.5	37.6	34.4	32.2	35.6	34.8	D	D	D	D	D	D
α	31.2	30.5	32.4	32.7	31.8	35.0	39.6	35.4	37.0	D	D	D	D	D	D
B	15.4	16.1	13.8	14.7	14.6	13.6	13.4	15.0	14.1	D	D	D	D	D	D
γ	15.1	15.1	15.6	16.1	16.0	16.9	14.8	14.0	14.1	D	D	D	D	D	D
Glob.	61.7	61.7	61.8	63.5	62.4	65.6	67.8	64.4	65.2	D	D	D	D	D	D
13024															
Alb.	41.7	42.7	42.7	40.9	42.0	39.7	35.4	35.4	36.5	38.1	37.6	36.0	36.7	35.1	35.2
α	26.4	27.7	29.3	30.1	25.9	31.0	31.2	33.6	34.5	28.8	31.6	28.6	27.4	30.2	33.8
B	17.1	13.7	13.9	13.8	16.7	13.7	19.3	17.0	14.4	19.4	17.1	17.3	18.7	18.2	14.6
γ	14.8	15.9	14.1	15.3	15.4	15.7	14.1	14.1	14.6	13.7	13.7	18.1	17.2	16.5	16.4
Glob.	58.3	57.3	57.3	59.1	58.0	60.3	64.6	64.6	63.5	61.9	62.4	64.0	63.3	64.9	64.8
13143															
Alb.	42.7	43.2	42.4	39.9	42.4	36.6	34.1	36.7	39.2	39.1	37.9	D	D	D	D
α	28.9	28.9	29.5	30.5	28.8	33.4	37.1	33.9	28.7	27.2	29.5	D	D	D	D
B	16.0	14.3	13.4	15.1	14.4	15.8	14.8	16.2	19.9	20.0	17.7	D	D	D	D
γ	12.4	13.5	14.6	14.5	14.4	14.1	14.0	13.3	12.3	13.8	14.9	D	D	D	D
Glob.	57.3	56.8	57.6	60.1	57.6	63.4	65.9	63.3	60.8	60.9	62.1	D	D	D	D

13144

Alb.	38.4	38.4	36.9	33.6	35.3	31.8	28.0	28.7	30.4	D	D	D	D	D	D
α	30.7	32.5	33.7	33.6	27.1	35.0	35.6	37.5	34.8	D	D	D	D	D	D
B	15.1	14.7	14.4	15.1	20.1	14.9	21.6	18.0	19.8	D	D	D	D	D	D
γ	15.9	14.4	15.0	17.7	17.6	18.4	14.7	15.8	15.1	D	D	D	D	D	D
Glob.	61.6	61.6	63.1	66.4	64.7	68.2	72.0	71.3	69.6	D	D	D	D	D	D

13145

Alb.	39.8	38.1	38.1	38.6	38.3	36.0	33.1	32.2	32.5	35.2	36.4	34.7	36.0	38.3	37.9
α	31.1	32.5	32.0	31.7	32.0	34.9	32.8	39.3	33.8	32.7	31.7	33.3	39.4	37.5	40.0
B	17.9	14.2	17.3	17.0	16.7	15.9	21.0	16.0	21.3	20.3	18.3	17.6	12.5	13.3	13.0
γ	11.2	15.2	12.7	12.7	13.1	13.2	13.2	12.5	12.3	11.8	13.6	14.4	14.0	10.9	9.1
Glob.	60.2	61.9	61.9	61.4	61.7	64.0	66.9	67.8	67.5	64.8	63.6	65.3	64.0	61.7	62.1

fractions are minor and should be considered for comparative purposes only.

Fluorescent Antibody Studies Results

Immunofluorescent examination of tonsil biopsies

Repeated sampling of tonsillar tissue was kept to a minimum due to the rapid onset, following biopsy, of a necrotic process which destroys considerable amounts of tissue adjacent to the biopsy site. This allowed a maximum of 5 samplings from an animal during the course of the disease. In view of our interest concerning the early pathogenesis of the disease and spread of the HCV, these biopsies were collected daily or three times weekly beginning the day of infection.

Viral antigen was detected much more quickly in the course of the disease produced by the virulent HCV-325 strain. The FATST, applied to tonsil biopsy sections collected 24 hours after infection with virulent HCV-325, revealed the presence of HCV antigen in all 5 animals of Group III. The lymphocytic tissue, particularly the germinal centers, contained fluorescing cells in all 5 animals. The crypt epithelium in the tonsil of animal 13136 also had small patches of cells which fluoresced at that time. Tonsil biopsies, collected from the same 5 animals 72 hours later, contained areas of immunofluorescence in the tonsillar surface epithelium, crypt epithelium and lymphocytic tissues. Immunofluorescence, once established in the tonsil, occasionally diminished in its intensity but

did not completely disappear. Tonsil biopsies collected from 4 of the 5 animals in Group I, infected with the same HCV-325, had no demonstrable immunofluorescence 18 hours after infection. A tonsil biopsy from animal 12825 had a few small areas of immunofluorescence in the germinal centers of the lymphocytic tissue. Forty-eight hours later strong immunofluorescence was observed in both surface and crypt epithelium and also in well defined groups of lymphocytes from all 5 animals.

Viral antigen was not detected in tonsil tissue from the animals of Groups II and IV, infected by the less virulent HCV-11325, prior to 7 days after infection. It is pertinent to point out that these biopsies were collected at 2 and 3 day intervals after infection, consequently antigen may have been present for 24 to 48 hours before it was observed. Strongest immunofluorescence was usually present first in the germinal centers of the lymphocytic tissue of the tonsil. In no case was the immunofluorescence detected in the epithelial tissue before it was observed in the germinal centers of the lymphocytic tissue. However, in several sections, particularly those collected 5 to 7 days after infection, immunofluorescence was observed in epithelium but not lymphocytes (Figure 16). Immunofluorescence in tonsils from animals of Groups II and IV, once it appeared, persisted until the animal died or the experiment was terminated.

Figure 16. Tonsil biopsy prepared by FATS technique showing true apple-green fluorescence due to fluorescein isothiocyanate which is conjugated to the anti-HCV antibody. This is the area most frequently infected in both crypt and surface tonsillar epithelium. 250 X

Immunofluorescent examinations of bone marrow aspirates

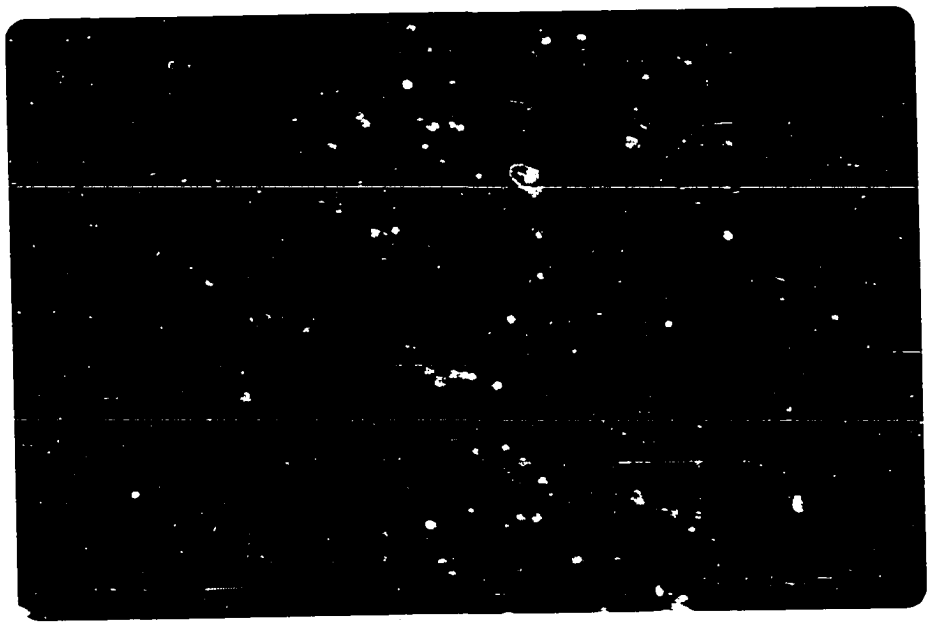
Cells of various sizes, ranging from some as large as rubriblasts and myeloblasts to others as small as mature segmented PMN leukocytes and metarubricytes, were present in the smears of bone marrow cells stained by FATST. Only the cytoplasm of cells appeared to contain the apple green fluorescence of fluorescein isothiocyanate - anti-HCV antibody conjugate (Figure 17). A granular yellow autofluorescence was present in the cytoplasm of some of the mature polymorphonuclear leukocytes (Figure 18).

Fluorescing cells were observed in the bone marrow material aspirated 24 hours after infection, from 2 of the 5 animals in Group I (Table 18). Bone marrow cells in the remaining 3 animals fluoresced 48 hours later. As is evident from Table 18, negative bone marrow samples were occasionally obtained from an animal which had previously been shown to be infected. This was apparently the result of the problems commonly associated with bone marrow aspiration, e.g., different cell populations are often sampled on consecutive aspirations, or a bone marrow sample may become contaminated by peripheral blood.

Infection by less virulent HCV-11325 was not detected in the bone marrow of pigs in Group II for 96 hours. Three of the 5 animals had small numbers of infected cells at that time. All five animals had moderate numbers of fluorescing cells in their bone marrow when samples were collected again 72 hours

Figure 17. Bone marrow smear stained by the FATS technique. The material was collected 72 hours after inoculation. Notice that a majority of cells fluorescing are small. 250X

Figure 18. Bone marrow smear prepared by FATS which shows the golden autofluorescence in the mature granulocytic leukocytes. 250X



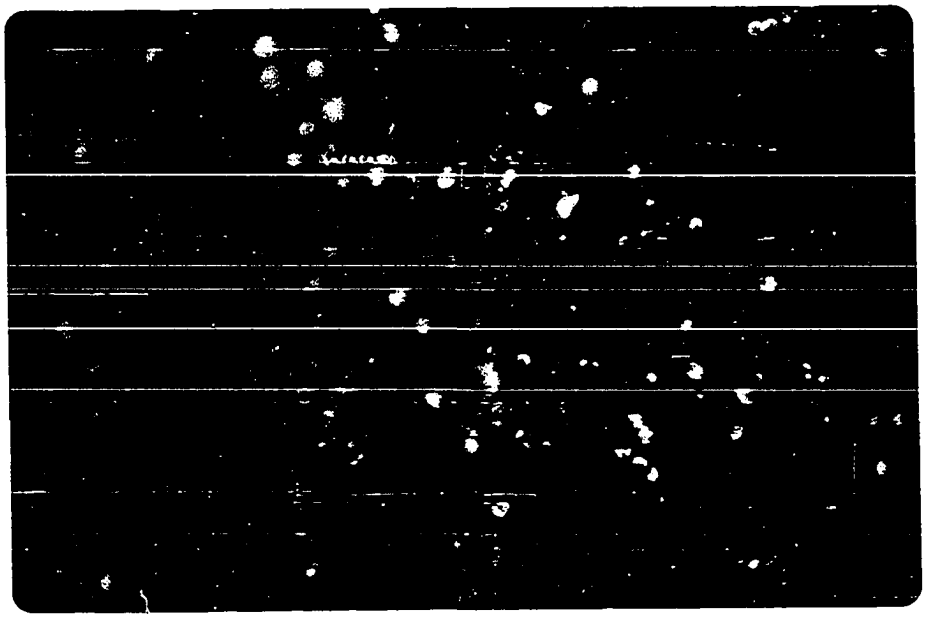


Table 18. Observation of immunofluorescence[a] in bone marrow cells of young pigs infected by virulent HCV-325 or less virulent HCV-11325

Date	8-25[b]	8-26	8-27	8-28	8-29	8-30
Group I						
12825	-	+	++	+++	+	++
12826	-	-	-	+++	++++	++
12827	-	-	-	+++	D[c]	D
12828	-	-	-	+++	++	D
12829	-	+	-	+++	++	++
Date	9-15[b]	9-17	9-19	9-22	9-24	9-26
Group II						
12941	-	-	-	++	+	+
12942	-	-	+	++	+	++
12943	-	-	+	+	+	+++
12944	-	-	+	+	++	+
12945	-	-	-	+++	+	+
Date	10-27[b]	10-28	10-29	10-30	10-31	11-1
Group III						
13136	-	+	+	+	++	++
13137	-	+	+	+	++	+
13138	-	-	-	+	+	D
13139	-	-	-	+	+++	+
13140	-	+	-	+	+++	+
Date	11-17[b]	11-19	11-21	11-24	11-26	11-28
Group IV						
13023	-	-	-	++++	+	+++
13024	-	-	-	++++	+++	++++
13143	-	-	-	+++	++	++
13144	-	-	-	+++	+	++
13145	-	-	-	++++	++	+++

[a]Symbols used to indicate proportion of bone marrow cells which were fluorescing: -,negative; +,less than 1 percent; ++, 1 to 10 percent; +++, 10 to 30 percent; ++++, more than 30 percent.

[b]Date of infection.

[c]D-animal died.

8-31	9-1	9-2	9-3			
+++	++	-	-			
D	D	D	D			
D	D	D	D			
D	D	D	D			
++	+++	-	+			
9-29	**10-1**	**10-3**	**10-6**	**10-8**	**10-10**	**10-13**
++	+++	++	+	+	+	+
+	+	D	D	D	D	D
+	++	-	D	D	D	D
++	+++	++	+	+	D	D
+	+	+	+	+	+	+
11-2	**11-3**	**11-4**	**11-5**	**11-6**		
+	++	+	D	D		
+	+	+	+++	+		
D	D	D	D	D		
+	+	D	D	D		
+	++	++	D	D		
12-1	**12-3**	**12-5**	**12-8**	**12-10**	**12-12**	
D	D	D	D	D	D	
+	+	++	++	+++	++++	
++	+	D	D	D	D	
D	D	D	D	D	D	
++	++	++	++	+++	++++	

later. Groups III and IV responded to infection, as would be expected, in much the same manner as Groups I and II respectively. Antigen of the more virulent HCV-325 was detected in the cytoplasm of the host pig's bone marrow cells approximately 144 hours earlier than HCV-11325 (Table 18). In the cells of pigs infected by the latter virus, however, a much larger number of the bone marrow cells fluoresced and with much greater intensity.

It was observed that cells of a variety of sizes had typical fluorescence early in the course of an infection. However, as the disease progressed, an increasingly larger proportion of the cells with fluorescing cytoplasm were the size of rubriblasts, myeloblasts, prorubricytes and promyelocytes (Figure 19). Fluoresing cells were most prevalent in the bone marrow material collected within one week after initial infection was observed. A gradual but consistent decline in numbers of fluorescing cells was then observed in the smears as the infection progressed. The changes described in this paragraph apply to the bone marrow cells in animals infected by both viral strains.

Immunofluorescent examination of tissues collected at necropsy

Viral antigen was detected in the tissues of 19 of the 20 animals in these studies (Table 19). Antigen was not detected in any of the tissues of animal 12945 in Group II. Immunofluorescence was observed in only tonsil and lymph node tissue

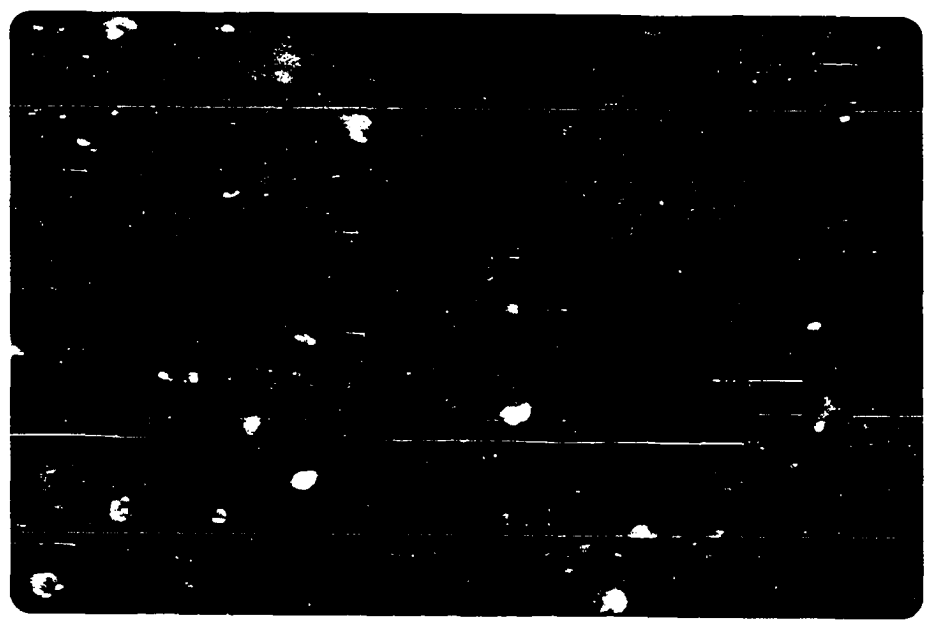


Figure 19. Bone marrow smear prepared by immunofluorescent technique. Compare the size of the fluorescing cells in this smear with Figure 17. Because of magnification differences, the best comparison is with the autofluorescing granulocytic leukocytes which have a granular, golden-yellow fluorescence. 430X

Table 19. Immunofluorescence[a] observed in tissues collected at necropsy from young pigs infected with a highly virulent (HCV-325) or moderately virulent (HCV-11325) strain of hog cholera virus

Group I HCV-325	12825	12826	12827	12828	12829
Tonsil	+++	+++	+++	++++	+++
Lymph node	++	+++	+++	+++	++
Spleen	+++	++	+++	++	++
Ileum	++	++	N[b]	+++	++
Group II HCV-11325	**12941**	**12942**	**12943**	**12944**	**12945**
Tonsil	++++	+++	+++	+++	-
Lymph node	+++	+++	++	+++	-
Spleen	++	+++	++++	++	-
Ileum	+++	+++	+++	++	-
Group III HCV-325	**13136**	**13137**	**13138**	**13139**	**13140**
Tonsil	++	++	+	+++	+++
Lymph node	+	+++	+++	+++	+++
Spleen	++	++	+	++	++
Ileum	++	++	+++	++	+++
Group IV HCV-11325	**13023**	**13024**	**13143**	**13144**	**13145**
Tonsil	+++	+++	++++	+++	+++
Lymph node	++	+++	++	+++	+
Spleen	+++	+++	++	+++	-
Ileum	++	+++	+++	+++	-

[a]Symbols used to indicate degree of fluorescence observed in the sections: -, negative; +, small patches or groups of cells; ++, 1 to 3 small cellular areas of fluorescence; +++, 4 to 9 distinct areas of fluorescence; ++++, extensive fluorescence in all parts of susceptible tissue.

[b]N-not collected.

of animal 13145 in Group IV. Both of these animals were infected by the less virulent HCV-11325. The small number of animals involved preclude any conclusions as regards the relationship of virulence of the HCV and its ability to establish and maintain itself in the tissues of its host.

The pattern of immunofluorescence observed in the tonsillar tissue collected at necropsy was similar to that observed in the biopsies. Although considerable subjectivity is involved in estimating the number of cells fluorescing and the intensity of the fluorescence present, some comparison between biopsy and necropsy tonsil tissue was attempted. Results of this comparison suggested that tonsillar tissue collected at necropsy had denser populations of fluorescing lymphocytes in the germinal centers than were observed in the biopsy material. The fluorescence observed in the crypt and surface epithelium was more patchy and uneven in biopsy tissues than in the necropsy material.

Both autofluorescence and pseudofluorescence were observed in the cells of the deepest layers of the surface and crypt epithelium. This seemed to be particularly troublesome in the tonsillar tissue from pigs with black skin. In general, the yellow color and granular consistency of the false reaction areas permitted differentiation; however, the use of alternate serial sections blocked by antisera was essential in questionable cases.

Serum Enzyme Assay

The serum enzyme assays were all completed with the exception of SGPT, which was not conducted after the early stages of the study. In this work, as well as in previously reported work (Cassidy and Seberg, 1967) and that of Compagnucci and Martone (1967), no significant change in the levels of SGPT were detected during the course of HC.

Serum alkaline phosphatase results

A gradual, but definite, decline in the SAP values was apparent in a majority of animals in 3 of 4 groups (Table 20). In Group I (virulent), 3 of 5 animals had elevated SAP values. In 2 of these animals the elevation was due to a several-fold increase which occurred within 24 hours prior to death. Four of the Group III (virulent) animals had SAP levels which elevated briefly for 24 to 72 hours after infection and then declined steadily until the animals died or were euthanatized. The fifth animal's SAP level rose to approximately twice its pre-infection level 96 hours after infection and the animal died 24 hours later. Nine of 10 animals infected by the less virulent HCV-11325 manifested SAP levels which declined steadily during the course of the disease. In a few of these animals a small transient increase in SAP levels occurred within a few days after infection. The major difference between the 2 groups infected by the 2 different viruses was the incidence of the persistent decline of SAP values in the animals of

Table 20. Serum alkaline phosphatase levels in blood of young pigs infected by a highly virulent (HCV-325) or less virulent (HCV-11325) hog cholera virus[a]

Date	Group I HCV-325 Animal numbers				
1969	12825	12826	12827	12828	12829
8-20	4.7	5.2	6.4	2.3	6.4
8-21	4.6	4.8	5.9	3.0	6.3
8-22[b]	4.6	4.4	5.4	3.1	6.4
8-25[b]	4.6	4.3	6.1	3.5	5.5
8-27	2.9	4.8	7.0	4.4	4.8
8-28	5.7	8.4	>40.0	14.2	7.1
8-29	4.2	5.9	D[c]	34.2	4.0
8-30	2.6	6.0	D	D	2.9
8-31	1.8	D	D	D	2.1
9-1	1.5	D	D	D	1.7
9-2	1.5	D	D	D	1.6
9-3	0.7	D	D	D	0.7
	Group II HCV-11325				
	12941	12942	12943	12944	12945
9-10	4.6	8.3	7.2	5.6	4.3
9-11	4.6	6.4	6.5	4.7	4.2
9-12[b]	5.2	6.1	4.4	6.3	5.0
9-15[b]	4.8	5.3	3.9	5.0	5.5
9-17	4.9	5.4	3.2	3.3	4.1
9-19	5.0	4.0	2.3	2.5	3.3
9-22	3.9	1.5	1.8	1.2	2.4
9-24	4.0	1.1	0.8	0.7	1.5
9-26	4.5	1.1	1.0	0.8	1.6
9-29	2.0	1.2	1.0	0.8	i.s.[d]
10-1	1.1	1.6	0.8	0.4	1.7
10-3	0.9	D	0.8	0.6	1.8
10-6	0.2	D	D	0.1	0.1
10-8	0.6	D	D	3.7	1.9
10-10	0.6	D	D	D	1.9
10-13	0.6	D	D	D	3.3
10-15	0.7	D	D	D	4.4

[a]Alkaline phosphatase reported in Sigma units.

[b]Date of infection.

[c]D-animal died.

[d]Insufficient sample.

Table 20 (Continued)

Date	Group III HCV-325 Animal numbers				
1969	13136	13137	13138	13139	13140
10-22	6.4	5.7	5.6	5.9	4.8
10-23	6.0	5.9	5.0	4.9	4.1
10-24[b]	5.5	5.3	4.7	4.9	3.9
10-27[b]	4.3	4.9	5.3	4.6	4.1
10-28	4.0	5.2	4.0	3.8	4.1
10-29	3.4	8.5	5.5	3.6	6.6
10-30	4.1	8.8	5.5	11.2	5.6
10-31	2.4	4.2	3.5	10.6	3.7
11-1	1.7	2.0	2.0	D	2.1
11-2	1.3	1.7	1.6	D	1.6
11-3	1.1	1.4	1.4	D	1.4
11-4	1.0	1.1	1.3	D	1.4
11-5	1.2	1.0	D	D	1.6
11-6	D	1.0	D	D	D
11-7	D	1.2	D	D	D
	Group IV HCV-11325				
	13023	13024	13143	13144	13145
11-12	7.0	6.3	6.0	6.9	7.8
11-13	5.4	5.7	5.8	4.8	6.1
11-14[b]	4.0	4.4	5.0	3.9	5.1
11-17[b]	3.9	3.8	3.6	3.6	4.2
11-19	3.6	3.7	4.0	3.8	3.5
11-21	2.6	2.8	2.4	3.1	3.7
11-24	2.1	1.9	1.7	2.0	1.8
11-26	1.6	1.2	1.4	1.2	1.4
11-28	1.1	0.7	1.1	0.7	0.7
12-1	D	0.5	0.6	D	0.8
12-3	D	0.6	0.9	D	0.7
12-5	D	0.6	D	D	0.7
12-8	D	0.4	D	D	0.5
12-10	D	0.6	D	D	0.7
12-12	D	0.5	D	D	0.9

Groups II and IV (less virulent).

Serum acid phosphatase results

Although some pronounced changes in the SAcP were present in the serum of animals from all 4 groups, the differences were less consistent in relation to groups of animals or the virulence of the infecting virus (Table 21). While 6 animals manifested a SAcP increase of varying degree, serum from 12 of them reflected a decline in the levels. Enzyme levels in serum of 80 percent of animals infected by HCV-11325 were reduced in the terminal stage. Sixty percent of the animals infected with HCV-325 had increased SAcP levels at the terminal stage of the disease. Serum acid phosphatase levels were increased in all animals of Group I and decreased in all animals of Group IV.

Serum glutamic oxaloacetic transaminase

A comparison of the SGOT levels in the infected animals of Groups I and II provides insight into the difference in extent of damage produced in a host by similar viruses of high and low virulence (Table 22). Within 72 hours SGOT levels in the animals of Group I, infected by virulent HCV-325, had increased from 6 to 30 times their pre-infection levels. With the exception of 1 animal, which had an elevated SGOT just prior to death, SGOT levels in animals of Group II were only moderately elevated. Post infection increases of SGOT observed in the animals of Group III when compared to those of Group I

Table 21. Serum acid phosphatase levels in blood of young pigs infected by a highly virulent (HCV-325) or less virulent (HCV-11325) hog cholera virus[a]

Date	Group I HCV-325 Animal numbers				
1969	12825	12826	12827	12828	12829
8-20	1.0	0.9	1.3	0.9	1.1
8-21	1.1	1.0	1.3	1.1	1.3
8-22[b]	1.2	0.9	1.2	1.1	1.4
8-25[b]	1.1	1.0	1.3	1.3	1.4
8-26	0.9	1.8	1.1	1.1	0.8
8-27	1.7	2.0	1.6	2.1	2.4
8-28	3.4	2.9	6.3	4.5	4.0
8-29	4.1	2.9	D[c]	7.8	5.0
8-30	3.1	2.4	D	D	4.3
8-31	2.0	D	D	D	3.0
9-1	1.2	D	D	D	1.8
9-2	0.9	D	D	D	1.3
9-3	1.5	D	D	D	1.3
	Group II HCV-11325				
	12941	12942	12943	12944	12945
9-10	1.5	2.0	1.4	1.6	1.6
9-11	1.5	1.8	1.7	1.5	1.6
9-12[b]	1.6	1.8	1.2	1.5	1.7
9-15[b]	1.8	1.8	1.1	1.6	2.1
9-17	1.8	1.8	1.1	0.3	1.9
9-19	1.5	1.6	0.7	1.1	1.8
9-22	1.5	1.9	1.0	1.4	1.8
9-24	1.5	1.3	1.0	1.0	1.6
9-26	1.7	1.7	0.7	0.8	1.5
9-29	1.5	1.6	0.6	0.6.	i.s.[d]
10-1	1.1	1.6	0.8	0.4	1.7
10-3	1.0	D	0.8	0.6	1.8
10-6	0.9	D	D	1.1	1.9
10-8	0.7	D	D	2.7	1.8
10-10	0.6	D	D	D	1.9
10-13	0.4	D	D	D	1.8
10-15	0.5	D	D	D	1.9

[a]Acid phosphatase reported in Sigma units.

[b]Date of infection.

[c]D-animal died.

[d]i.s.-insufficient sample.

Table 21 (Continued)

Date	Group III HCV-325 Animal numbers				
1969	13136	13137	13138	13139	13140
10-22	2.0	2.2	1.5	2.1	1.7
10-23	2.2	2.0	1.6	2.0	1.5
10-24[b]	2.1	1.9	1.7	1.8	1.6
10-27[b]	1.5	1.9	1.8	2.0	1.5
10-28	1.2	1.7	1.4	1.3	1.3
10-29	1.8	3.1	2.4	1.8	2.3
10-30	2.9	3.8	4.3	3.1	3.0
10-31	3.0	3.8	5.4	4.3	3.1
11-1	2.8	2.6	5.2	D	2.5
11-2	1.8	1.5	3.3	D	1.5
11-3	1.2	1.0	1.7	D	1.0
11-4	0.9	0.7	1.1	D	0.9
11-5	0.9	0.6	D	D	0.8
11-6	D	0.5	D	D	D
11-7	D	0.6	D	D	D
	Group IV HCV-11325				
	13023	13024	13143	13144	13145
11-12	1.8	1.7	1.8	1.5	2.1
11-13	1.0	1.1	1.2	0.8	1.2
11-14[b]	1.0	1.1	1.3	0.8	1.3
11-17[b]	1.2	1.2	1.2	1.1	1.6
11-19	1.0	1.2	1.0	1.0	1.3
11-21	1.4	1.5	1.3	1.5	1.6
11-24	2.1	1.9	1.6	1.7	1.7
11-26	1.3	1.5	1.1	1.2	1.4
11-28	0.8	1.1	0.8	0.9	1.0
12-1	D	0.7	0.4	D	0.9
12-3	D	0.5	0.6	D	0.5
12-5	D	0.4	D	D	0.4
12-8	D	0.4	D	D	0.4
12-10	D	0.7	D	D	0.3
12-12	D	0.4	D	D	0.5

Table 22. Serum glutamic oxaloacetic transaminase levels in blood of young pigs infected by a highly virulent (HCV-325) or less virulent (HCV-11325) hog cholera virus[a]

Date	Group I HCV-325 Animal numbers				
1969	12825	12826	12827	12828	12829
8-20	18	25	21	21	12
8-21	23	28	25	33	41
8-22[b]	30	21	30	26	35
8-25[b]	26	23	33	21	39
8-26	26	41	20	26	21
8-27	66	60	86	55	94
8-28	153	206	1000	216	187
8-29	136	195	D[c]	i.s.[d]	118
8-30	180	300	D	D	141
8-31	166	D	D	D	153
9-1	159	D	D	D	118
9-2	154	D	D	D	108
9-3	99	D	D	D	92
	Group II HCV-11325				
	12941	12942	12943	12944	12945
9-10	21	31	28	25	26
9-11	23	33	26	20	20
9-12[b]	20	26	15	26	24
9-15[b]	17	24	17	17	43
9-17	16	25	18	17	21
9-19	17	37	21	32	18
9-22	15	50	37	35	32
9-24	16	57	33	33	41
9-26	26	52	32	50	37
9-29	28	75	39	60	i.s.
10-1	26	86	63	68	33
10-3	24	D	43	60	32
10-6	28	D	D	72	28
10-8	35	D	D	159	20
10-10	32	D	D	D	20
10-13	30	D	D	D	23
10-15	30	D	D	D	28

[a]Glutamic oxaloacetic transaminase reported in Sigma-Frankel units.

[b]Date of infection.

[c]D-animal died.

[d]i.s.-insufficient sample.

Table 22 (Continued)

Date	Group III HCV-325 Animal numbers				
1969	13136	13137	13138	13139	13140
10-22	45	37	26	23	26
10-23	33	26	25	21	21
10-24[b]	37	33	26	21	25
10-27[b]	25	30	33	21	23
10-28	57	30	43	20	23
10-29	82	216	108	55	173
10-30	153	315	173	196	135
10-31	130	180	147	206	130
11-1	119	114	114	D	99
11-2	90	75	104	D	78
11-3	90	57	124	D	63
11-4	75	48	108	D	82
11-5	147	54	D	D	166
11-6	D	52	D	D	D
11-7	D	94	D	D	D
	Group IV HCV-11325				
	13023	13024	13143	13144	13145
11-12	37	25	43	28	30
11-13	28	20	37	28	26
11-14[b]	23	17	35	18	23
11-17[b]	26	16	26	20	21
11-19	30	21	35	30	21
11-21	37	37	46	33	32
11-24	135	63	82	75	57
11-26	141	130	141	159	54
11-28	82	103	75	68	37
12-1	D	57	33	D	47
12-3	D	54	113	D	47
12-5	D	54	D	D	41
12-8	D	20	D	D	21
12-10	D	35	D	D	18
12-12	D	18	D	D	79

exemplify the variation in response of a single species of animal to one particular viral pathogen. The effect of the additional virulence of HCV-325 over that of HCV-11325 is further evident when the SGOT levels of Groups I and III are compared with those of Groups II and IV.

Serum lactic dehydrogenase

In the preceding paragraph, the extent to which serum enzyme levels are elevated was discussed in relationship to the virulence of the HCV. In the case of LDH values, the greater virulence of the HCV may be apparent in the uniformity and consistency with which the hosts were affected. In all ten animals infected with virulent HCV-325, the LDH levels were elevated at least 5-fold. Once established, elevated serum LDH values were recorded from 3 to 8 days or until death occurred (Table 23). In comparison, of 10 animals infected by less virulent HCV-11325 only 4 had levels of 2000 units or higher. All 4 animals with elevated LDH levels died during the course of the disease. Differences in LDH values between groups of animals infected by the same virus were not as marked as they were in the case of SGOT values.

Clinical and Necropsy Studies

Response of the 20 animals to infection by one of the 2 HCV strains was assessed, in addition to hematology, by clinical, necropsy and microscopic examinations.

Table 23. Serum lactic dehydrogenase levels in blood of young pigs infected by a highly virulent (HCV-325) or less virulent (HCV-11325) hog cholera virus[a]

Date	Group I HCV-325 Animal numbers				
1969	12825	12826	12827	12828	12829
8-20	1315	1325	1100	1200	1190
8-21	1190	1000	1040	1235	1235
8-22[b]	1270	1150	1040	1290	1380
8-25[b]	1215	1000	1085	1150	1190
8-26	1535	910	1015	1130	850
8-27	1815	1165	1215	1225	1645
8-28	7300	5500	8600	6450	7900
8-29	9150	7350	D[c]	11000	8500
8-30	7425	7850	D	D	6200
8-31	6125	D	D	D	1815
9-1	5875	D	D	D	1870
9-2	5575	D	D	D	1630
9-3	1965	D	D	D	1605
	Group II HCV-11325				
	12941	12942	12943	12944	12945
9-10	1410	1390	1400	1325	1180
9-11	1305	1215	1285	1290	1140
9-12[b]	1335	1015	1040	1215	1100
9-15[b]	1175	1140	990	1130	1360
9-17	1140	1055	895	1015	895
9-19	1000	1125	840	1200	960
9-22	950	1335	1000	1000	795
9-24	1175	1675	1140	1025	1485
9-26	1040	1615	925	1390	1515[d]
9-29	990	6300	1345	5475	i.s.
10-1	1140	8150	6950	5900	1240
10-3	975	D	5825	5575	1200
10-6	1380	D	D	2000	1510
10-8	1360	D	D	8350	1465
10-10	1530	D	D	D	1055
10-13	1400	D	D	D	925
10-15	1325	D	D	D	935

[a]Lactic dehydrogenase reported in Berger-Broida units.

[b]Date of infection.

[c]D-animal died.

[d]i.s.-insufficient sample.

Table 23 (Continued)

Date	Group III HCV-325 Animal numbers				
1969	13136	13137	13138	13139	13140
10-22	1085	1230	1070	1180	1040
10-23	1125	1125	1165	1085	1100
10-24[b]	1190	1265	1240	950	950
10-27[b]	1085	1070	1370	975	935
10-28	1115	1025	910	780	780
10-29	1345	7475	1940	1215	1380
10-30	8450	8900	8900	9000	6525
10-31	7425	8925	9250	8950	5775
11-1	5775	6575	6350	D	1960
11-2	1870	1870	7150	D	4875
11-3	6725	6675	7475	D	7525
11-4	1935	2000	7375	D	7650
11-5	6350	5350	D	D	9075
11-6	D	1910	D	D	D
11-7	D	6250	D	D	D
	Group IV HCV-11325				
	13023	13024	13143	13144	13145
11-12	1200	1015	1215	1100	1200
11-13	1115	1000	1085	935	895
11-14[b]	910	895	950	925	810
11-17[b]	895	870	885	855	885
11-19	870	855	780	855	715
11-21	960	885	935	795	910
11-24	1760	1605	1530	1125	1725
11-26	1850	1560	1380	1420	1605
11-28	1590	1450	1200	1430	1140
12-1	D	1115	885	D	1390
12-3	D	1295	5350	D	1315
12-5	D	1410	D	D	1560
12-8	D	1270	D	D	1295
12-10	D	1760	D	D	1175
12-12	D	1115	D	D	1740

Clinical

Body temperatures are recorded in Table 24. They indicate that body temperature elevation occurs concurrently with the other changes described previously. Whereas the virulent HCV-325 produced changes, including fever, as early as 24 hours after inoculation, HCV-11325 required a minimum of 48 hours to effect the same changes.

Anorexia, inactivity and a general malaise was evident in the animals as soon as the body temperature became elevated. Five to 15 days after infection, a crusty exudate was present in the conjunctiva. This frequently became viscous and, in a few cases, caused the animal to have difficulty in opening it's eyes. The orbital bleeding technique did not seem to affect this condition adversely. A similar exudate was present, transiently, around the nostrils of a few animals in each group. It was particularly severe in the Group III animals. Blotchy purplish areas appeared in the skin of white pigs. This was particularly noticeable in pigs which had survived infection for 2 weeks or more. The majority of the animals developed constipation and, later, diarrhea. Animals infected with virulent HCV-325 that died shortly after infection did not appear to develop diarrhea. Although several animals manifested generalized weakness and incoordination at various times in the course of the disease, frank convulsions and other signs suggestive of central nervous system involvement were not seen during the daily observation periods. Death was in-

variably preceded by a drop in body temperature.

Necropsy

Most of the animals in the 4 groups had several lesions associated with HC. Petechial hemorrhages were common in the kidneys, urinary bladder, coronary band of the heart, and skin. Ecchymotic hemorrhages were particularly evident in skin on the medial sides of the legs and on the abdomen. Lymph nodes were severely congested or hemorrhagic to the extent they resembled small blood clots. Necrotic lesions were present in the tonsil of every animal. A majority of these lesions were associated with the tonsil biopsy sites. Pneumonia varying in extent from a small plum-colored area on the tip of one lobe of the lung to a severe fibrinous pneumonia enveloping the anterior half of both lungs was present in approximately 20 percent of the animals. Splenic infarcts were present in 60 percent of the animals. The encrusted circular raised lesions in the large intestine called "button ulcers" were observed in about 30 percent of the animals. Bone changes attributable to HC were observed in ribs of three animals. A meticulous examination of orbits of the animals, following enucleation, revealed only a trace of blood in the areas where the periorbital venous plexus had been opened for sample collection the day of necropsy.

One animal of Group I (12826) had a severe bilateral hydronephrosis, bilateral dilatation of both ureters, a hemor-

rhagic cystitis and a severely dilatated urethra. Animal 12827 of Group I was moderately icteric when necropsied 3 days after being infected with HCV-325. A severe congestion of the spleen and lesions typical of HC were also present. A purulent pyelonephritis was present in the right kidney of animal 12828.

One animal of Group II had a bilateral dilatation of both ureters and another's urethra was dilatated distal to the bladder.

Four of 5 animals in Group III had moderate to severe dilatation present in one or more of the following structures: urethra, urinary bladder and ureters. In one of the 4 animals a moderately severe bilateral hydronephrosis was present.

In Group IV, 2 animals apparently died of extensive hemorrhage. Due to the thrombocytopenia commonly present in it, HC cannot be ruled out as at least one causative factor in the death of these 2 animals. Animal 13143 apparently died due to blood loss. A large blood clot which filled a major part of the stomach and extended up the esophagus and protruded into the pharynx was observed at necropsy. Large amounts of dark clotted blood were also present in the large intestine. The second animal (13023) had a very large blood clot in the peritoneal cavity which extended from the diaphragm over the stomach, enveloping the spleen and part of the small intestine (Figure 20). Approximately 400 ml. of dark red bloody fluid were present in the peritoneal cavity. Careful dissection disclosed the primary source of the hemorrhage to be in the

Figure 20. Animal 13023 with massive hemorrhage extending from the diaphragm around the stomach, enveloping the spleen and part of the small intestine. Source was the uterus

uterus.

Histopathology

Microscopic examination confirmed the lesions identified at necropsy. In addition, a marked vasculitis and other encephalitic changes were evident in brain tissue of all 20 animals. In a few animals, modest changes typical of HC were observed in the epiphyseal line region or ribs.

Hog Cholera Viruses

Isolation of viruses

Isolation and identification of virus from all 10 animals of Groups I and III infected by virulent HCV-325 was accomplished by the FA tissue culture technique (FATCT). Viral isolation from 3 of the 5 animals of Group II and all 5 of those in Group IV infected by HCV-11325 was also successful. All viral isolations were performed by the Swine Viruses Unit of Diagnostic Services, NADL, Ames, Iowa, under the direction of Dr. W. C. Stewart.

Table 24. Body temperatures of young pigs infected by a highly virulent hog cholera virus (HCV-325) or one of less virulence (HCV-11325)[a]

Date	Group I HCV-325 Animal numbers				
1969	12825	12826	12827	12828	12829
8-20 AM	103.8	104.0	104.2	104.0	103.0
8-20 PM	103.4	103.2	103.6	104.0	103.4
8-21 AM	102.6	103.2	103.2	103.6	103.2
8-21 PM	104.0	103.8	103.6	103.6	103.2
8-22 AM	102.6	102.8	103.0	103.2	102.4
8-22 PM	103.2	103.2	103.4	103.4	103.2
8-23	102.4	102.5	102.4	103.0	102.4
8-24	103.2	103.2	104.2	103.0	103.4
8-25 AM[b]	102.6	102.0	103.2	102.4	103.0
8-25 PM	103.6	103.4	103.4	103.2	103.4
8-26 AM	105.8	106.2	106.4	105.8	106.4
8-26 PM	105.4	106.6	105.2	105.0	106.0
8-27 AM	106.6	106.2	106.0	106.8	106.4
8-27 PM	106.8	106.4	105.8	106.4	107.0
8-28 AM	106.2	105.2	105.6	106.6	106.2
8-28 PM	106.0	104.4	D[c]	104.8	105.4
8-29 AM	106.6	105.2	D	100.0	104.4
8-29 PM	106.6	104.6	D	104.0	106.4
8-30	107.0	105.0	D	D	106.4
8-31	106.0	D	D	D	106.6
9-1	105.6	D	D	D	105.0
9-2 AM	105.0	D	D	D	105.6
9-2 PM	104.1	D	D	D	104.4
9-3 AM	104.4	D	D	D	104.2
9-3 PM	104.2	D	D	D	104.0
9-4	103.6	D	D	D	104.2
	Group II HCV-11325				
	12941	12942	12943	12944	12945
9-10 AM	103.0	104.0	103.6	102.4	102.4
9-10 PM	103.2	103.0	103.4	104.0	103.4
9-11 AM	101.8	101.6	101.8	102.4	102.4
9-11 PM	104.0	104.2	104.6	103.4	103.2
9-12 AM	102.2	102.2	102.8	102.0	102.8
9-12 PM	103.2	103.6	103.4	102.6	103.0

[a]Temperature reported in degrees Fahrenheit.

[b]Date of infection.

[c]D-animal died.

Table 24 (Continued)

Date	Group II HCV-11325 (Continued) Animal numbers				
1969	12941	12942	12943	12944	12945
9-13	102.5	102.4	103.0	102.2	103.0
9-14	102.4	102.0	102.2	102.6	103.0
9-15 AM[b]	102.4	101.8	102.4	102.2	101.8
9-15 PM	103.4	103.6	103.4	103.8	104.4
9-16 AM	102.6	101.2	102.2	102.8	102.8
9-16 PM	103.2	102.8	103.6	103.4	103.6
9-17 AM	102.8	101.8	102.8	103.4	103.2
9-17 PM	102.8	103.2	104.4	104.2	103.8
9-18 AM	102.4	103.8	106.0	104.4	104.6
9-19 AM	103.0	105.8	106.0	105.4	104.0
9-19 PM	103.0	105.8	106.6	106.4	105.8
9-20	104.0	106.0	106.8	106.0	105.4
9-21	102.2	106.6	104.8	106.4	105.0
9-22 AM	102.0	106.4	106.6	106.8	104.4
9-22 PM	102.6	106.0	106.2	106.4	104.8
9-23 AM	103.2	107.0	107.6	106.8	104.6
9-23 PM	102.4	108.0	107.4	108.2	104.6
9-24 AM	102.2	106.4	106.8	106.0	105.0
9-24 PM	103.6	107.6	106.6	106.8	105.2
9-25 AM	104.6	105.8	107.8	107.4	104.8
9-25 PM	103.4	106.4	105.2	106.6	105.4
9-26 AM	103.8	105.2	106.4	106.8	104.0
9-26 PM	103.6	106.6	107.4	105.4	105.8
9-27	104.6	105.6	107.7	106.0	105.0
9-28	104.2	105.0	106.4	105.6	104.4
9-29 AM	106.4	105.4	105.8	106.0	104.8
9-29 PM	107.2	104.6	106.4	106.2	105.6
9-30 AM	104.6	104.1	106.8	106.6	104.3
9-30 PM	106.4	105.4	105.6	107.0	104.6
10-1 AM	105.4	102.6	106.0	104.8	104.2
10-1 PM	105.0	103.8	103.6	104.8	104.2
10-2 AM	106.8	102.2	104.6	105.8	103.6
10-2 PM	106.8	D	105.0	105.6	104.0
10-3 AM	106.6	D	103.8	104.6	103.2
10-3 PM	105.6	D	104.2	104.4	103.8
10-4	105.6	D	103.2	104.2	103.8
10-5	105.0	D	101.8	104.4	102.2
10-6 AM	105.8	D	D	104.6	102.0
10-6 PM	106.0	D	D	105.4	104.2
10-7 AM	105.8	D	D	103.6	104.0
10-7 PM	106.0	D	D	103.4	103.6
10-8 AM	104.8	D	D	103.2	102.2
10-8 PM	104.8	D	D	103.6	103.6
10-9 AM	104.0	D	D	D	103.2

Table 24 (Continued)

Date	Group II HCV-11325 (Continued) Animal numbers				
1969	12941	12942	12943	12944	12945
10-9 PM	105.0	D	D	D	103.2
10-10 AM	104.6	D	D	D	103.5
10-10 PM	103.0	D	D	D	103.4
10-11	104.2	D	D	D	103.6
10-12	104.4	D	D	D	103.4
10-13 AM	105.6	D	D	D	103.8
10-13 PM	105.2	D	D	D	104.0
10-14 AM	104.8	D	D	D	104.2
10-14 PM	104.8	D	D	D	103.0
10-15	104.6	D	D	D	102.6
	Group III HCV-325				
	13136	13137	13138	13139	13940
10-21 AM	103.3	103.6	102.2	103.2	103.0
10-22 AM	102.8	102.6	103.0	103.0	103.2
10-22 PM	103.0	102.4	103.0	102.6	103.8
10-23 AM	102.6	102.6	102.2	102.0	102.6
10-23 PM	102.4	102.8	102.6	102.4	103.2
10-24 AM	102.4	101.8	102.0	102.2	101.8
10-24 PM	102.4	102.8	102.8	103.2	103.8
10-25	102.6	102.0	102.8	103.4	103.6
10-26	102.4	102.6	102.2	103.2	103.4
10-27 AM[b]	102.4	102.6	101.4	101.4	101.6
10-27 PM	103.4	102.8	103.2	102.6	102.8
10-28 AM	105.4	104.2	106.8	105.0	104.4
10-28 PM	105.6	106.0	105.8	105.4	104.6
10-29 AM	107.0	106.4	105.2	105.6	105.6
10-29 PM	107.0	107.0	106.2	106.0	106.4
10-30 AM	106.8	105.4	106.0	104.0	105.8
10-30 PM	106.4	105.0	106.4	104.2	105.6
10-31 AM	106.4	105.4	107.2	104.6	107.8
10-31 PM	107.2	105.0	106.8	105.2	106.8
11-1	106.6	106.2	106.8	D	105.0
11-2	106.2	105.8	103.0	D	104.6
11-3 AM	104.4	105.4	104.2	D	104.2
11-3 PM	105.0	105.8	104.0	D	104.8
11-4 AM	104.4	105.2	103.0	D	105.0
11-4 PM	104.4	104.4	101.0	D	103.8
11-5 AM	105.0[d]	103.8[d]	D	D	100.8
11-5 PM	N[d]	N[d]	D	D	D
11-6	D	105.0	D	D	D
11-7	D	104.6	D	D	D

[d]N-temperature not taken.

Table 24 (Continued)

Date	Group IV HCV-11325				
1969	13023	13024	13143	13144	13145
11-12 AM	102.0	102.4	103.2	103.4	102.4
11-13 AM	103.4	103.8	103.4	103.4	102.8
11-13 PM	103.6	104.0	103.6	103.8	103.8
11-14 AM	102.2	102.0	103.0	102.4	102.6
11-14 PM	103.0	103.6	104.0	103.6	103.4
11-15	102.2	102.0	102.8	102.6	102.4
11-16	102.9	102.6	103.2	102.4	102.5
11-17 AM[b]	102.4	103.2	103.2	102.2	103.0
11-17 PM	103.0	103.6	103.4	103.2	102.8
11-18 AM	103.4	103.8	103.6	102.4	103.0
11-18 PM	103.6	103.6	103.8	103.8	103.4
11-19 AM	103.6	103.6	103.4	103.6	103.4
11-19 PM	103.4	103.8	103.4	103.8	103.2
11-20	105.0	105.0	105.8	105.4	104.4
11-21 AM	106.2	106.4	106.0	106.0	105.4
11-21 PM	106.8	106.8	107.0	107.2	106.2
11-22	107.2	108.0	107.1	106.2	106.0
11-23	106.4	106.5	106.8	106.0	105.0
11-24 AM	107.6	106.2	107.4	106.6	106.4
11-24 PM	106.8	106.4	107.4	106.8	106.8
11-25 AM	106.0	107.0	106.4	105.6	106.0
11-25 PM	106.4	107.2	106.0	105.4	106.0
11-26 AM	107.2	106.8	105.8	106.8	104.2
11-26 PM	104.6	104.2	106.2	107.6	105.6
11-27 AM	105.4	106.5	105.5	106.4	107.0
11-27 PM	105.6	106.4	106.2	106.0	107.2
11-28 AM	106.0	106.4	104.4	104.4	106.6
11-28 PM	105.6	105.4	105.8	D	106.2
11-29	D	105.7	104.8	D	105.4
11-30	D	106.1	105.0	D	105.7
12-1 AM	D	105.6	103.4	D	105.0
12-1 PM	D	105.4	102.0	D	105.4
12-2 AM	D	105.2	103.4	D	105.8
12-2 PM	D	106.0	103.6	D	106.2
12-3 AM	D	105.8	91.0	D	105.6
12-3 PM	D	105.4	90.8	D	105.2
12-4 AM	D	105.8	D	D	105.8
12-4 PM	D	105.8	D	D	105.4
12-5 AM	D	105.4	D	D	104.4
12-5 PM	D	105.6	D	D	104.4
12-6	D	105.2	D	D	104.6
12-7	D	105.5	D	D	104.2

Table 24 (Continued)

Date	Group IV HCV-11325 (Continued) Animal numbers				
1969	13023	13024	13143	13144	13145
12-8 AM	D	104.5	D	D	104.3
12-8 PM	D	104.0	D	D	104.2
12-9 AM	D	105.4	D	D	103.2
12-9 PM	D	105.8	D	D	104.0
12-10 AM	D	106.0	D	D	103.8
12-10 PM	D	104.8	D	D	103.8
12-11 AM	D	104.4	D	D	105.2
12-11 PM	D	105.8	D	D	106.0
12-12	D	104.6	D	D	104.4

DISCUSSION

Hematologic Examinations

Erythrocyte sedimentation rate

Method comparison study Comparison of the 3 methods for determining the ESR of swine blood gave results similar to those of Gambino et al. (1965) who worked with human blood. The modified Westergren method resulted in the least variation between samples, or the greatest statistical precision. Gambino et al. (1965) modified the classical Westergren method by replacing the 3.8 percent sodium citrate solution, which served the dual purposes of blood diluent and anticoagulant, with 0.85 percent saline. Use of the modified procedure requires collection of blood in an appropriate anticoagulant, preferably tripotassium ethylenediamine tetra-acetate monohydrate (K_3EDTA). Apparently, diluted blood is essential for accurate determination of the ESR of swine or human blood. Dawson (1960) published work pointing out that narrow bore tubes such as the Westergren ESR tube caused excessive aggregation of erythrocytes unless the plasma proteins are diluted. In order to avoid such undesirable effects, the sedimentation tube should have a bore greater than 4 square millimeters (mm^2). To conserve blood both the Wintrobe and Westergren tubes have bores considerably narrower than 4 mm^2. There appear to be 2 physical reasons for the reduced accuracy of the Wintrobe tube. First, it uses undiluted blood in a tube

with too narrow a bore. Second, the Wintrobe tube is too short and consequently the free fall time is too short. Wintrobe (1967) has acknowledged that his method gives more misleading results than the Westergren technique and attributes this chiefly to the narrow bore of the tube. The modified Westergren method, as demonstrated by the results of these studies, would appear to be the method of choice for determining the ESR of swine blood.

Modified Westergren versus dilute Wintrobe method Results of this trial conclusively demonstrate that the modified Westergren method was adequately sensitive to detect deviations in the ESR due to infection by HCV. It is noteworthy that ESR values underwent change as early in the course of the disease as body temperature and TWBC count. Also the relative increase of the ESR was generally several-fold greater than the reduction in TWBC numbers or elevation in body temperature. This suggests that on a unit basis, the ESR provides the most sensitive indicator of blood component injury due to HC.

This trial also provided a comparison between the Westergren and Wintrobe methods when diluted blood was used in both tubes. Gambino et al. (1965), in comparing 32 diluted human blood sample ESR's measured in both Wintrobe and Westergren tubes, reported a closer correlation of values in the normal range than when the modified Westergren and Wintrobe methods with undiluted blood were used. He also reported that in the higher ESR ranges, regardless of whether the blood was diluted

or not, the Wintrobe tube gave lower values than modified Westergren method. This general trend is present in the data of Table 4 which suggests that even with dilute blood, use of the Wintrobe tube results in a less sensitive measurement.

Erythrocyte sedimentation rate of experimental animals

The ESR is a simple test used routinely as an index of active disease of many types. In order to discuss the changes occurring in the ESR during HC infection, a brief explanation of the underlying mechanism, to the extent that it is understood, will be given here. The test is based on the phenomenon that erythrocytes in blood to which an anticoagulant has been added will sediment into a packed column in the lower part of the tube or container. The rate of this process is dependent on several factors, chief of which are rouleaux formation, concentration of fibrinogen in the plasma and concentration of alpha and beta globulins in the plasma or serum. Red blood cells sediment because of their density exceeds that of the plasma. Opposing this is the buoyancy-producing effect of the plasma. The strength of this opposing force is directly proportional to the surface area of the erythrocytes. Aggregation of several erythrocytes in the form of a cylindrical column produces a "rouleaux" with much less surface area than the total surface area of the individual RBC's. The result is that the force of the plasma opposing sedimentation is proportionately reduced. Rouleaux formation is the greatest single factor leading to an increased sedimentation rate. It is increased by

plasma colloid changes resulting mainly from increased concentrations of fibrinogen and alpha and beta globulins. The relationship of the fibrinogen concentration of plasma to the ESR is generally a linear one. Gamma globulins have little effect on the ESR (Lynch et al., 1969). Plasma albumin tends to reduce the ESR by retarding the attraction between the RBC's. Alpha and beta globulin levels also have a marked effect on the ESR. Increased levels almost always produce an elevation of the ESR. Most acute infections are characterized by reduced plasma albumin and increased alpha globulins and fibrinogen, a combination which enhances the ESR (Lynch et al., 1969).

One remarkable difference between the responses of the 2 groups of animals infected by the 2 HC viruses of different degrees of virulence, was their ESR response in the course of the disease. In every animal infected with the more virulent HCV-325, a noticeable increase occurred in the ESR within 24 hours after inoculation. This elevation persisted for varying lengths of time and then dropped to or near pre-infection levels. After a variable length of time, the ESR increased again and persisted at elevated levels until the animals died or the experiment was terminated. Plasma fibrinogen concentration increases very rapidly in acute diseases with lesions throughout the body, such as in HC. It is quite probable that this was the major cause of the initial elevation in ESR observed in these animals. A rise in fibrinogen was not recorded

in the electrophoretograms made during this study, as serum, not plasma, was the source of proteins. Frequently after blood cells had been separated and discarded, the serum would form a clot. It seems logical to assume that this was due to an excess of fibrinogen, however, the possibility of a delayed clotting mechanism must also be considered.

The second rise in ESR is at least partially explained by the results of electrophoretic separation of serum proteins. Between the time of inoculation and the beginning of the second rise in ESR, the serum globulins increased an average of 6 grams percent. This suggests that the ESR and electrophoresis results obtained from pigs of Groups I and III (virulent) can be explained on the basis of the mechanisms determining the ESR. Although less dramatic and obvious, the elevation of the ESR in the swine of Groups II and IV was accompanied by a gradual increase in serum globulins. Between the time of inoculation of the less virulent HCV-11325 and the start of the ESR increase, the serum globulins increased an average of 5.4 percent. One explanation for the absence of a biphasic response in the ESR of these animals may have been that the virus was not producing adequate damage to the host to produce a sufficient elevation in the fibrinogen level of the plasma. Another possibility is that the accelerated ESR due to increased fibrinogen, if it occurred, was maintained by the macroglobulin produced by the immune mechanism stimulated by the HCV.

Weide and Twiehaus (1959) reported an increased ESR in young pigs 8 days after they were inoculated with lapinized, hog cholera vaccine virus. Our findings are also in agreement with Sippel (1958) who reported a consistent increase in the ESR of HC infected swine.

Packed cell volume, hemoglobin concentration and total red blood cell numbers

Due to their intimate relationship to each other, these 3 measurements are discussed together. Their responses in the HCV infected animals paralleled each other, an observation which is in agreement with that of Sorensen et al. (1961).

Although several viruses affecting animals are known to produce an anemia, e.g., infectious equine anemia and infectious canine hepatitis viruses, HCV has not been commonly incriminated as possessing this property. Data in Tables 7, 8 and 9 indicate a reduction in PCV, Hgb and TRBC within 24 to 48 hours after inoculation of HCV. A satisfactory explanation for this phenomenon has not been available up to this time. Sirbu et al. (1964) using FA techniques described HCV antigen on the surface of erythrocytes from infected animals.

Although these reduced RBC's and related values have been reported since Dinwiddie (1914) described the anemia of HC, they have received minimal attention. A possible explanation is that the relative decrease in these values is not as profound in relation to their totals as is the change in total leukocyte numbers. It must also be noted that there are few

if any pathologic changes associated with HC which resemble those of a hemolytic anemia, such as that seen in porcine eperythrozoonosis.

The changes observed in bone marrow in this study, and discussed later, indicate that HCV enters and possibly replicates in cells of both the erythroid and myelocytic cell series. This apparently greatly reduces the number of young blood cells available for replacement of senile red and white blood cells in the circulation. The RBC has a much longer life span than most leukocytes. Using carbon-14, Bush et al. (1955) determined a mean lifetime span for the pig erythrocyte of 62 days while Jensen et al. (1956), working with iron-59, calculated a value of 63 days and Hansard and Kincaid (1956) obtained a value of 71 days using chromium-51. No precise data on the life span of the porcine leukocytes was encountered in the literature. Patt and Maloney (1959), using tritiated thymidine, were able to estimate the life span of canine neutrophilic granulocytes at between 8 to 14 days. Current research on human lymphocytes with radioactive markers has revealed 2 types of lymphocytes, one with a life span of 2 to 3 days, another which survives for 200 to 300 days and possibly even for years (Lynch et al., 1969). The difference in the life spans of these cells suggests a hypothesis that might explain the differences in the relative degrees of damage suffered by the populations of RBC's and WBC's. Because of their much longer life span, the need for mature red blood cells would be much

less than that for granulocytes or short-lived lymphocytes. In addition, the affinity of the HCV for leukocytes would result in still greater degrees of relative reduction in TWBC numbers. The largest number of HCV infected leukocytes apparently are observed in the peripheral blood within 48 hours after infection (Mihajlovic et al., 1969). Solorzano (1962) has reported the presence of HCV in leukocytes and published the first procedure for demonstrating HCV antigen in these and other tissue cells.

Blood loss due to sample collection is one consideration which merits discussion. Approximately 15 ml. of blood were collected daily from each animal on the days listed in the data tables. Kornegay (1967) removed 12 ml. of blood twice daily for 5 days from 9, 14 kilogram pigs. Hemoglobin values followed PCV values which decreased from 34.9 to 31.3 percent. In the same study he removed 12 ml. of blood from pigs of similar age, weight and sex for a period of 100 days. Hemoglobin concentration declined from 10.8 to 9.6 grams percent and the PCV values dropped from 35 to 31 percent. These results suggest that at least some of the decreases observed in these observations are possibly due to blood loss from sample collection. It may also be pertinent to point out that no declines in PCV, Hgb and TRBC levels occurred during the 5-day pre-infection period when blood samples were collected at the same time intervals. To further support the contention that these changes are due at least in part to the HCV, one

has only to refer to the numerous reports of anemia in both field and laboratory studies of HC. Among the more pertinent are those of King and Wilson (1910), Lewis et al. (1914), Lewis and Shope (1929a), Kernkamp (1939), and Weide and Twiehaus (1959). The design of this experiment was not appropriate for the solution of the question of the extent to which bleeding for samples produced the decline in these values; however, it cannot be entirely disregarded.

In summary, results of this work and the cited reports strongly suggest that HCV strains, irregardless of their virulence, produce damage to the erythroid cell series of the bone marrow which is reflected in reduced PCV, Hgb and TRBC levels in the peripheral blood.

Red blood cell indices

The size and hemoglobin content of erythrocytes are defined by erythrocytic indices calculated from results of determinations of PCV, Hgb and TRBC numbers. Normal values for the pig are for MCV, a mean of 63 cubic microns with a range of 50 to 68; for MCHC, a mean of 32 percent with a range of 30 to 34; for MCH, a range of 16.6 to 22.0 micromicrograms (Benjamin, 1961).

Mean corpuscular volume The extreme fluctuations of this index value before, as well as after, infection prevented the detection of any meaningful and consistent trend in the change of size of the erythrocytes. Due to this circumstance

it was not possible to recognize any variation from normal values which might be due to HCV. With the exception of animals 12825 and 12827, which had consistently low MCV values, the animals were all in the normal range.

Mean corpuscular hemoglobin There was no perceptible, consistent change in this value in the animals of the 4 groups, which did not occur before infection as well as after exposure to HCV. No deviations of significance between groups of animals infected by the 2 HCV's of different degrees of virulence were apparent.

Mean corpuscular hemoglobin concentration While minor elevations or decreases in the MCHC occurred at various stages of the HC infection, no consistent changes were detected in this index value among the 20 animals. The uniformity of the MCHC values, all within the normal range, is noteworthy.

It is quite interesting that anemias due to bone marrow depression causing decreased blood formation are characterized as normocytic and normochromic. Normal MCV and MCHC values of this study identify the anemia as normocytic and normochromic. These findings together with those of immunofluorescent examination of bone marrow and peripheral TWBC and TRBC counts are compatible with bone marrow injury due to HCV.

Reticulocytes

The normal level of reticulocytes in swine blood is as high as 2 percent and the time required for additional reticu-

locytes to appear in the blood following acute and severe blood loss is around 4 days (Schalm, 1965). These values on replacement times are for the bovine, as figures for swine could not be found in literature. Life span of reticulocytes is considered to be no longer than a few days in the bovine.

Results of the reticulocyte counts further support the contention that the erythrocytic bone marrow cell series is damaged by HCV. With few exceptions, following infection, reticulocyte numbers did not regain their pre-infection levels. There was no perceptible difference attributable to the different strains of HCV infecting the animals. The exceptions, mentioned above, were single animals with elevated levels observed for 1 or 2 days. Animal 12945 of Group II was apparently effecting a recovery from HC and the increased reticulocyte levels reflect a compensatory replacement effort.

The consistently reduced levels of reticulocytes observed in these animals, for periods of several weeks or longer, probably reflect a persistent infection of the bone marrow. This speculation was confirmed by immunofluorescent studies of bone marrow cells collected at previously described intervals.

Nucleated red blood cells

Following the transient rise in numbers of nucleated RBC's in the animals of Groups I and III, there was an abrupt reduction and disappearance of these cells. In Groups II and IV no sharp increase was observed in numbers of nucleated RBC's.

However, by the middle and late stages of infection with HCV-11325, nucleated RBC's were no longer present in smears of peripheral blood.

We are unable to explain the marked difference in response of these immature erythrocytes in animals infected by the two viruses. Additional support for the contention that the erythron elements of the bone marrow are damaged by the HCV, is present in the disappearance of the nucleated RBC's from the peripheral blood following infection of the animals in all 4 groups.

Total white blood cell counts

The uniformity with which the 2 strains of HCV produced depression of both RBC and WBC numbers within rather narrow postinoculation time limits, is noteworthy. Equally important is the difference in time of development of leukopenias produced in the animals infected by the 2 viruses. This latter observation is in agreement with that of Sorensen et al. (1961).

Little question remains as to the etiology of the leukopenia, considered to be a classical finding in HCV infected pigs. Results of this study and others (Solorzano, 1962, and Sirbu et al., 1964) place the site of leukocyte destruction in both the bone marrow and peripheral blood. Hog cholera virus has been isolated from bone marrow by Pehl and Schulze (1958). Boynton (1946) has been able to produce in vitro

replication of HCV in bone marrow cell cultures derived from susceptible animals. In a part of our work to be discussed later, HCV antigen was consistently demonstrated in the cytoplasm of cells present in smears of bone marrow material taken from the animals of all four groups. Solorzano (1962) and Mihajlovic et al. (1969) have demonstrated HCV antigen by immunofluorescent techniques, in blood leukocytes taken from HCV infected pigs. Dunne et al. (1958) were able to obtain 61 serial passages of HCV in porcine leukocyte cultures. Loan and Gustafson (1964) have also demonstrated that HCV may persist for up to 2 years in cultures of subcultural swine buffy coat cells. In both cases the HCV was virulent for susceptible pigs after serial passage. Strain HCV-11325 used in these studies was isolated by means of swine blood buffy coat cell cultures after cultivation on conventional porcine kidney cell cultures failed to detect the virus. These facts strongly suggest that the HCV produces the leukopenia by entering into the leukocytes of the bone marrow and peripheral blood, utilizing their metabolic machinery for its replication and ultimately destroying the cells. Weide et al. (1962), using gnotobiotic and mono-contaminated pigs, demonstrated that the leukopenia present in HC was due to the HCV. Similar studies in the gnotobiotic dog with canine distemper virus by Gibson et al. (1965) and with gnotobiotic cats using feline infectious enteritis virus by Rohovsky and Griesemer (1967), have also demonstrated that the leukopenias in these diseases are of viral

origin.

Differential leukocyte counts

Contradiction and variation exist between reports describing the differential leukocyte counts from both normal and HC affected swine. Results of this study were also characterized by a lack of uniformity. Swine differential leukocyte counts, as suggested by data from this study and previously published reports, King and Wilson (1910), Kernkamp (1939) and Weide and Twiehaus (1959), are too variable both before and after HCV infection to provide reliable information on the disease.

Bone marrow smears

A careful study and classification of 300 cells in each of 262 individual bone marrow samples was carried out. Samples were collected at previously described times from all 20 animals. Unfortunately, bone marrow aspirate material frequently contains only a small and not necessarily representative sample. All types and techniques of bone marrow aspiration or biopsy are open to this shortcoming. Consequently bone marrow material cannot be evaluated from a quantitative standpoint. During our examinations no unusual cell types or other abnormalities were observed in postinfection smears that were not present in smears collected prior to inoculation of the HCV.

One significant item of information obtained from the examination of bone marrow smears was the myeloid:erythroid ratio. Lahey et al. (1952) published the figure 1.77 ± 0.52

as the M:E ratio for bone marrow of young pigs. Table 15 contains the M:E ratios obtained from results of each bone marrow examination. Marked fluctuations are present in these values, even within the same animal from day to day. If mean values of M:E ratios from each sample collected before and after infection are considered, the latter values will obviously be much higher. Increased M:E ratios are generally the product of either a hyperplasia of myelocytic bone marrow with the same or reduced number of erythroid cells or unchanged numbers of myelocytic cells with a reduced number of erythroid cells. In view of the leukopenic blood counts on peripheral blood, collected from the same animal and at the same time as the bone marrow material, the elevated M:E ratios are probably due to a reduction of the erythrocytic cell precursors in the bone marrow due to the HCV.

Serum Protein Studies

Total serum proteins

A majority of the animals infected by the more virulent HCV-325 showed a slight increase in serum protein levels. An opposite effect was evident in serum from animals infected by the less virulent HCV-11325. In most cases the difference between serum protein levels in the first and last samples was less than the fluctuations observed daily. These findings are not in agreement with those of Kostyunina and Bychkov (1965) who reported a decrease in total proteins during the

first 6 days after the injection of HCV. Poul (1962) reported a 20 percent increase in total serum proteins following hyper-immunization of immune swine with HCV. Konopatkin (1962) also reported an increase in total serum proteins in vaccinated pigs after they were challenged with a virulent HCV.

Electrophoretic separation of serum proteins

An obvious trend was apparent in separation patterns produced by electrophoresis of the serum proteins from the 20 pigs. It was characterized by a decrease in albumin and increase in globulins. According to Vesselinovitch (1955), this dysproteinemia, in which albumin decreases and globulins increase, is a manifestation of the animal to any kind of stress and is a sign of the "adaptation syndrome". These results are in agreement with those of Weide and King (1962), Kostyunina and Bychkov (1965) and Mengeling and Packer (1969). A consistent elevation of the alpha globulin was observed in these studies. Similar findings have been reported by several workers (Weide and King, 1962; Konopatkin, 1962; Mengeling and Packer, 1969). Kostyunina and Bychkov (1965) reported that alpha globulins did not change during the first 6 days after infection with HCV.

The difference in their effects on the gamma globulin levels of their hosts, produced by the 2 HCV's, probably reflects their difference in virulence (Mengeling and Cheville, 1968). While 30 percent of the animals infected with the

milder HCV-11325 had depressed gamma globulin levels, 60 percent of those infected by HCV-325 had reduced levels of the same fraction. An accurate comparison of the results of this investigation with published findings would not be valid because of the variety of electrophoretic techniques and apparatus which were used. Equally important to keep in mind is the fact that the changes in the various protein fractions are relatively small and comparisons such as those made here are frequently of questionable validity.

Immunofluorescent Studies

Fluorescent examination of tonsil biopsies

Findings from this study are in agreement with those of Aiken et al. (1964) who reported the detection of HCV antigen in tonsil sections as early as 72 hours postinoculation. Detection was accomplished by use of the FATS technique. The earlier detection, at 24 hours postinoculation, of the HCV antigen in our work may have been due to one or more factors: (1) HCV-325 is a highly virulent virus capable of replicating very rapidly, (2) host animals in this study were specific-pathogen-free (SPF) hog cholera susceptible pigs from herds which have been shown to be highly susceptible to this strain of HCV, (3) the technical personnel in the laboratory where these studies were conducted, have the detection of HCV antigen in swine tissues as one of their major responsibilities and consequently have further developed and highly refined the

FATS technique. Aiken et al. (1964) and Teebken et al. (1967) also reported that modified and attenuated HC viruses were identified in tonsillar tissues 5 to 9 days postinoculation. This is in agreement with findings with the less virulent strain HCV-11325. Antigen of this virus was not detected in FATST stained tonsillar tissue before 7 days postinoculation.

Observations by Teebken et al. (1967) are not in complete agreement with those made in this study. In their studies they described patterns by which the fluorescence developed following infection of pigs by HCV's of different degrees of virulence. They described immunofluorescence in the tonsillar crypt epithelium of pigs infected 48 hours previously by a highly virulent virus. Seventy-two hours postinoculation reticuloendothelial cells in diffuse lymphoid tissue were showing fluorescence. Our observations were almost the opposite in that cells in germinal centers fluoresced prior to epithelial cells. Teebken et al. (1967) reported that immunofluorescence due to hog cholera vaccine virus was confined primarily to plaque-like areas of epithelial cells lining the deep areas of tonsillar crypts. In our work the HCV of lower virulence behaved essentially like the highly virulent one except for the longer incubation period. Any comparison of the attenuated vaccine HCV used by Teebken et al. (1967) and our HCV-11325 is obviously not appropriate. They also reported that inactivated HCV vaccines produced an atypical granular

fluorescence in the germinal centers only, 4 to 7 days after injection of the vaccine. A yellow-colored, granular fluorescence in the germinal centers of tonsillar tissue from normal pigs that have had no experience of HCV antigen of any type was commonly observed. One major difference in the 2 studies was that Teebken et al. (1967) killed their animals for each examination, while our work is based on biopsies collected at various intervals from 5 animals at a time. Consequently, it seems our work would provide a more accurate chronological picture of the sequence of events occurring in the pathogenesis of HCV infection in the swine tonsil. Kubin and Kölbl (1969), have been able to detect HCV in FATS stained lymph node sections as early as 2 days after infection.

Immunofluorescence observed in bone marrow aspirates

No previously published reports of the FATST study of bone marrow were encountered in the literature. Sorensen et al. (1961) described a hypoplasia of bone marrow as being present in HCV infected pigs, however, immunofluorescence was not used in their work. Almost every cell in many of the bone marrow smears examined by immunofluorescence contained HCV antigen. This finding was always confined to animals which had been infected for 5 days or longer. The prevalence of infected cells in a typical bone marrow smear is evident in Figures 17 and 18. A comparison of a similar smear stained by conventional techniques leaves no doubt that large numbers of both erythroid and myelocytic precursors are infected with

HCV.

The demonstration of HCV antigen in cytoplasm of bone marrow cells from animals infected 24 hours previously with HCV-325 is interesting in view of the findings of Pehl and Schulze (1958). These workers isolated HCV from the lymphatic and blood vessels of pigs infected 15 to 24 hours earlier. They were not able to isolate it from a variety of tissues including bone marrow until 48 hours after infection. If the animals and techniques of Pehl and Schulze (1958) were compatible with those of this study, a span of 24 hours exists between the time HCV antigens were demonstrated by the FATS method and infectious HC virions were isolated. Work with various viruses, particularly the picorna viruses (Watanabe et al., 1962), on virion replication strongly suggests that viral capsid protein pools may exist prior to assembly of intact virions. If this were the case, our examination of bone marrow material by immunofluorescence may have demonstrated only HCV capsid protein, or the soluble antigen of HCV (Ritchie and Fernelius, 1967, 1968) and capsid protein or the soluble antigen alone. Although it is beyond the scope of this work, a fairly simple and practical solution seems feasible. Aspiration of bone marrow from HCV infected animals and then division of the sample for concurrent virus isolation and examination of the bone marrow smear by FATST should resolve the question of whether the immunofluorescence is due to infective virions or their antigenic components in the cells.

Immunofluorescent examination of tissues collected at necropsy

Specific fluorescence was demonstrated in 19 of 20 animals. The absence of immunofluorescence in tissues of animal 12945 is consistent with hematologic findings which indicate the animal was recovering from HC. Animal 13145 which was infected with HCV-11325, as was animal 12945, also appeared to be recovering from HC. Mengeling and Cheville (1968) reported that in the first and third phases of chronic HC, produced by a mildly virulent HCV (HCV-331), the HCV antigen was distributed in a general manner in the lymphocytic and epithelial tissues. During their second phase of the disease they reported that the viral antigen was concentrated primarily in epithelial tissues. Aiken et al. (1964) reported that HCV antigen from attenuated HC vaccine viruses was identified by the FATST in the tonsils only. Apparently our moderately virulent HCV-11325 still retains the spreading and replicative characteristics of its more virulent relative HCV-325, as the length of time required for the activities to be completed was the major difference between the 2 strains.

The affinity of the HCV for lymphocytic tissues in all parts of the pig's body and its destructive effect on them probably accounts for a part of the lymphopenia observed in HC.

Serum Enzyme Assay Procedures

Serum alkaline phosphatase

Two distinct patterns of change in the serum alkaline phosphatase levels were evident in the 4 groups of animals. Elevated levels were observed in pigs which became severely ill and died. A consistent decline in SAP was observed in those pigs which lived 10 days or longer. The gradual decline of alkaline phosphatase which normally occurs in young animals (Benjamin, 1961) should be kept in mind while evaluating this decline. The high levels of SAP in young animals are correlated with bone formation. Osteoblasts are thought to release alkaline phosphatase into the blood during bone formation (Benjamin, 1961). In most of the animals a small decline in the SAP levels prior to infection was present (Table 20). The change was probably due to maturation. Statistical analysis of data detected significant differences between preinfection and postinfection means of the SAP values from all groups. Significant differences were present from the first or second day after infection until the termination of the experiment.

Alkaline phosphatase is present in the intestine, kidney, placenta, liver cells, leukocytes and osteoblast cells of the bone (Lynch et al., 1969). Alkaline phosphatase of the serum has at least three different isoenzyme components. Separation of isoenzymes, usually by electrophoresis, frequently permits

location of the tissue or origin of an elevated serum value (Lynch et al., 1969). In this case, such a procedure would permit assigning an increased level of total SAP to lesions of one of the several tissues listed above (Benjamin, 1961).

Marked serum alkaline phosphatase level increases which occurred just prior to death, or during severe phases of the disease, were probably due to extensive damage to several of the tissues containing significant amounts of alkaline phosphatase. Mengeling and Cheville (1968) and Mengeling and Packer (1969), have shown that HCV is present throughout the body only during certain periods of chronic HC. They reported generalized localization of virus in tissues of HC animals in the early and late stages of the disease. These periods would coincide with the periods when SAP in our animals was most frequently elevated.

The consistent decline in SAP levels in the majority of the animals is quite unique. The condition of hypophosphatasia, an inherited genetic disorder of human infants, is the only condition which we are aware of where very low serum alkaline phosphatase levels are present. Nutritional and other osteodystrophies are characterized by normal or elevated SAP values.

The following hypothesis which seems consistent with the above facts is offered as a possible explanation. Hog cholera virus, by destroying or inhibiting the osteoblasts of bone, which are hyperactive in animals of this age, produces the

reduced alkaline phosphatase levels observed in infected animals. Dunne (1964a) considers the interruption of bone growth at the epiphyseal line of the costochondral junction as a lesion of growing pigs, which occurs as frequently in HC infected pigs as any other lesion. Marked elevations of SAP produced by widespread, acute infections would mask the effect on the osteoblasts and lowered SAP levels. Conclusive proof of this hypothesis would require the histochemical demonstration of changes in alkaline phosphatase concentration in osteoblasts at the epiphyseal line of rapidly growing bones of young pigs. Concurrent SAP isoenzyme separation and characterization would be used to determine which SAP fraction was elevated. These would be conducted in conjunction with total SAP, serum calcium and serum phosphorus assays. Disturbances of metabolism of the latter 2 elements are also present according to Dunne (1964a) during HC.

Serum acid phosphatase

Statistical analyses of the data from the SAcP assays were no more informative than direct examination and comparison of the data. Pre-infection levels ranged from 0.8 to 2.2 Sigma units for the 20 animals. Postinfection levels varied from 0.3 to 7.8 Sigma units. These ranges and inconsistencies following infection make the drawing of conclusions difficult.

The original decision to assay this serum enzyme was largely motivated by the publication of Cheville and Mengeling

(1969) concerning the pathogenesis of hog cholera. They described the presence of markedly increased numbers of lysosomes in cells containing viral antigen. Acid phosphatase is one of the numerous enzymes present in lysosomes. The acid phosphatase histochemical reaction is commonly used to demonstrate lysosomes. It was felt that assays of SAcP would reveal if the lysosomal membranes had been stabilized by HCV or some other phenomenon was permitting survival of the lysosomes. Although 12 of the 20 animals reflected slightly reduced SAcP values over the course of the disease, the reductions were insufficient to warrant any definite conclusions relative to the report of Cheville and Mengeling (1969).

Serum glutamic oxaloacetic transaminase

In the pig SGOT is found in almost all tissues and consequently elevated levels are not organ specific. Increased levels are observed in a variety of pathologic states in which disease occurs. The results of this study indicate that SGOT levels serve as accurate indicators of tissue injury which occurs in animals infected by virulent strains of HCV. Cornelius et al. (1959) published SGOT levels of 27.3 ± 7.8 Sigma-Frankel units for normal swine. In myocardial infarction of man the degree of elevation of the enzyme level is roughly proportional to the extent of the damage. Elevated SGOT levels have been described in swine affected by erysipelas by Dougherty et al. (1965) and Witzel et al. (1967); in swine influenza

by Šlesingr (1965); and in liver and muscular dystrophy by Orstadius et al. (1959). Compagnucci (1966), and later Compagnucci and Martone (1967) in Italy, and Cassidy and Seberg (1967) in the United States, reported elevated SGOT levels in serum from HCV infected pigs. Cassidy and Seberg (1967) were unable to demonstrate elevation of serum enzymes in animals infected by HCV's of low virulence. These reports suggest that SGOT determinations would not be of great value in the differential diagnosis of HC or in pathogenesis studies of that disease.

Serum lactic dehydrogenase

Because of the universal distribution of this enzyme in tissues and blood cells, measurements of the total activity of LDH probably best serve only as an indicator of an active disease process present in the patient. Localization of the tissue source of an elevation of the total LDH is effected by the isoenzyme procedure mentioned previously.

Significant differences in group means are present in LDH values from animals of Groups I and III (virulent) from the third day after infection until the eighth and ninth days respectively. In comparison, similar procedures applied to data from Groups II and IV (less virulent) revealed significant differences after infection, only on days 14, 16, 18 and 23 in Group II and on the sixteenth day in Group IV.

Such findings suggest that serum LDH provides an accurate

indication of the disease process in swine. However, it appears that LDH in conjunction with SGOT assays would provide an even more sensitive indication of the active disease process in HC affected pigs. A comparison of Table 22 and Table 23 reveal the remarkable resemblance in the profiles produced by fluctuations of the serum enzyme levels in response to HCV infection by the virulent strain of virus.

Experimental Animals

Clinical studies

Clinical responses, in all 20 animals, to the HCV with which they were infected, were typical of those observed in previously studied cases of the disease caused by highly and moderately virulent HCV strains. They were not typical of cases of HC produced by the HCV strains of low virulence described by Sorensen et al. (1961). In infections produced by HCV's of low virulence, frequently the only evidence that animals have been infected is the immunity they possess when challenged by more virulent strains of HCV.

Necropsy findings and histopathology studies

The lesions, observed both grossly and microscopically were identical with those observed in animals experimentally infected with strains of HCV of similar degrees of virulence (Cassidy and Seberg, 1967). They also were similar to those described by Dunne (1964a).

These findings support the assumption that the animals experienced characteristic HC. This tends to sustain the validity of the results from our hematologic and immunofluorescent studies.

Hog Cholera Viruses

Isolation of viruses

Isolation of HCV from the splenic tissue of at least 90 percent of the infected animals, is to be expected in those situations where the tissue to be examined for the virus is removed within a few minutes to a few hours after death. In those cases where the tissues could not be processed for tissue culture inoculation immediately, the tissues were frozen at -20 C as soon as possible. A possible explanation for the lack of isolation of virus from animals 12944 and 12945 is present in the report by Mengeling and Packer (1969). These authors were unable to isolate HCV from spleens 10 days after inoculation of HCV of low virulence. They also were unable to isolate HCV from the spleens until 14 days after inoculation of a HCV of moderate virulence. This suggests that the high levels of gamma globulin which Mengeling and Cheville (1968) and Mengeling and Packer (1969), described as probably interfering with HCV isolation may have been involved in the unsuccessful isolation attempts reported here.

Detection of viral antigen

The detection by the FATST of HCV antigen in 19 of the 20 animals is in close agreement with the report of Kubin and Kölbl (1968). These workers were able to detect HCV antigen with the FATST in all of the experimentally infected animals from which HCV was isolated by tissue culture techniques.

SUMMARY

Hematologic and immunofluorescent methods were used to study the pathogenesis of HCV infection in the hemopoietic organs of 9 to 11-week-old pigs. Ten animals were infected with the highly virulent (HCV-325) strain of hog cholera virus and 10 others with a less virulent strain (HCV-11325) of virus. Changes produced in the animals infected with the 2 strains were compared. The major difference observed was that with the more virulent virus less time elapsed between inoculation of virus and detection of the viral antigen and hematologic changes produced by the virus.

A comparative study of 3 methods for determining the erythrocyte sedimentation rate of swine blood was conducted. A modification of the Westergren method was selected because it had high statistical precision and was the most sensitive to changes in the erythrocyte sedimentation rate produced in hog cholera. Rates for pigs 1 to 2 months old were: mean 2.81 and standard error of 3.79 millimeters per hour; for 3-month-old pigs the mean was 2.36 and standard error 0.15 millimeters per hour. Infection with the more virulent hog cholera virus produced elevated erythrocyte sedimentation rates within 24 hours while the less virulent virus required 48 hours to produce the same change. A biphasic rate increase pattern followed in the animals infected by the more virulent virus while a consistent elevation occurred in those infected with

the less virulent virus.

A consistent decline in packed cell volume, hemoglobin concentration and total red blood cell numbers occurred at approximately the same time as the erythrocyte sedimentation rate increased. The three red blood cell indices, mean corpuscular volume, mean corpuscular hemoglobin concentration and mean corpuscular hemoglobin, indicated the presence of a normocytic, normochromic anemia in the infected animals. A marked reduction in reticulocyte and nucleated red blood cell numbers occurred after infection by both strains of virus. These findings were all considered to reflect the injury to the bone marrow produced by the 2 hog cholera viruses.

A severe drop in leukocyte numbers occurred within 24 hours in animals inoculated with highly virulent HCV-325, and within 144 hours in animals injected with the less virulent HCV-11325.

Alterations in bone marrow cell morphology, due to HC, were not detected. Myeloid:erythroid ratios became elevated sporadically after infection. This was attributed to a reduction in erythroid cells, rather than hyperplasia of myelocytic elements, in view of the concurrent leukopenia present in the peripheral blood.

Total serum protein determinations did not disclose any significant changes following infection. Electrophoretograms derived from the same serum proteins demonstrated a moderate decrease in albumin and increase in total globulins.

Viral antigen was detected by immunofluorescence in tonsillar tissue of pigs 24 hours after infection with HCV-325 and 48 hours after inoculation of HCV-11325. Immunofluorescence persisted in tonsils until the animals died or the experiment was terminated. In bone marrow material studied by immunofluorescent technique the 2 viruses were detected at approximately the same times as they were observed in the tonsillar tissues. Immunofluorescence was present in selected tissues collected at necropsy in 19 of the 20 animals.

Enzyme studies indicated that in hog cholera infected animals alkaline phosphatase levels are reduced and serum glutamic oxaloacetic transaminase and serum lactic dehydrogenase are increased. Acid phosphatase levels did not change remarkably during the course of the disease. The sensitivity of serum glutamic oxaloacetic transaminase and lactic dehydrogenase levels as indicators of tissue damage due to virulent hog cholera virus are discussed.

Clinical, necropsy and histopathologic studies of infected animals demonstrated changes characteristic of hog cholera caused by viruses of high and moderate virulence. Hog cholera virus was isolated from 18 of the 20 animals used in the study.

These studies demonstrate that HCV inflicts extensive damage to the hemopoietic organs of infected swine. Both erythroid and myelocytic cells in the bone marrow are damaged and destroyed. Subsequently a progressive anemia and absolute neutropenia, eosinopenia and lymphopenia are present. The

number of animals which recover from these effects is inversely proportional to the virulence of the infecting hog cholera virus.

LITERATURE CITED

Aiken, J. M., Hoopes, K. H., Rhodes, M. B. and Twiehaus, M. J. 1967. Nonspecificity of fluorescent-antibody test for distinguishing hog cholera virus strains. J. Am. Vet. Med. Assoc. 150: 59-61

Aiken, J. M., Hoopes, K. H. and Stair, E. L. 1964. Rapid diagnosis of hog cholera: a direct fluorescent-antibody technique. Am. Vet. Med. Assoc. Proc. 101: 282-284.

Albritton, E. C. 1952. Standard values in blood. Philadelphia, Pa., W. B. Saunders Co.

Andersch, M. A. and Szczypinski, A. J. 1947. Use of p-nitrophenyl phosphate as the substrate in determination of acid phosphatase. Am. J. Clin. Pathol. 17: 571-574.

Armed Forces Institute of Pathology. 1968. Manual of histologic and special staining techniques. 3rd ed. New York, N.Y., McGraw-Hill Book Co.

Atherton, I. K. 1923. Hog cholera control versus prevention. J. Am. Vet. Med. Assoc. 17: 278.

Bedell, D., McDaniel, H. A., Clark, C. D., Grey, A. P. and Aiken, J. M. 1969. Recommended minimum standards for detecting hog cholera viral antigen by the fluorescent antibody tissue section technique. U.S. Animal Health Assoc. Proc. 73: 440-443.

Benjamin, M. M. 1961. Outline of veterinary clinical pathology. 2nd ed. Ames, Iowa, Iowa State University Press.

Bessey, O. A., Lowry, O. H. and Brock, M. J. 1946. A method for the rapid determination of alkaline phosphatase with five cubic millimeters of serum. J. Biol. Chem. 164: 321-329.

Boynton, W. H. 1946. Preliminary report on the propagation of hog cholera virus in vitro. Vet. Med. 41: 346-347.

Bunce, S. A. 1954. Observations on the blood sedimentation rate and the packed cell volume of some domestic farm animals. Brit. Vet. J. 110: 322-328.

Bush, J. A., Berlin, N. I., Jensen, W. N., Brill, A. B., Cartwright, G. E. and Wintrobe, M. M. 1955. Erythrocyte life span in growing swine as determined by glycine-2-C^{14}. J. Exp. Med. 101: 451-455.

Cabaud, P. G., Wroblewski, F. and Ruggiero, V. 1958. Colorimetric measurement of lactic dehydrogenase activity of body fluids. Am. J. Clin. Pathol. 30: 234-236.

Cahill, E. A. 1929. Post-vaccination trouble - a possible diagnostic method. J. Am. Vet. Med. Assoc. 74: 425-431.

Calhoun, L. M. and Smith, E. M. 1964. Hematology and hematopoietic organs. In Dunne, H. W., ed. Diseases of swine. 2nd ed. Pp. 37-61. Ames, Iowa, Iowa State University Press.

Cameron, H. S. 1947. Immunologic and clinical studies on plasma fractions from domestic livestock. I. The antibody content of porcine and bovine gamma globulin. Am. J. Vet. Research 8: 153-156.

Carle, B. N. and Dewhirst, W. H. 1942. A method for bleeding swine. J. Am. Vet. Med. Assoc. 101: 495-496.

Cartwright, G. E., Fay, J., Tatting, B. and Wintrobe, M. M. 1948. Pteroylglutamic acid deficiency in swine; effects of treatment with pteroylglutamic acid, liver extract and protein. J. Lab. Clin. Med. 33: 397-416.

Cartwright, G. E., Palmer, J., Hitchings, G., Elion, G., Gunn, F. and Wintrobe, M. M. 1950. Studies of the effect of 2,6-diaminopurine on the blood and bone marrow of swine. J. Lab. Clin. Med. 35: 518.

Cartwright, G. E., Tatting, B., Ashenbrucker, H. and Wintrobe, M. M. 1949. Experimental production of a nutritional macrocytic anemia in swine. Blood 4: 301-323.

Cassidy, D. R. and Seberg, S. A. 1967. Studies of serum enzymes in swine infected by two strains of hog cholera virus. Develop. Studies Conducted Diagnostic Serv., Natl. Animal Disease Lab., Fiscal year 1967. Agr. Research Serv. Bull. 91-70: 18-22.

Cherry, W. B., Goldman, M. and Carski, T. R. 1960. Fluorescent antibody techniques in the diagnosis of communicable diseases. Public Health Service Pub. 729.

Cheville, N. F. and Mengeling, W. L. 1969. The pathogenesis of chronic hog cholera. Lab. Invest. 20: 261-274.

Coffin, D. L. 1945. Manual of veterinary clinical pathology. Ithaca, N.Y., Comstock Publishing Co.

Cole, C. G. 1932. Leucocyte counts on the blood of normal, cholera-infected and recently immunized pigs. J. Am. Vet. Med. Assoc. 81: 392-400.

Compagnucci, M. 1966. Preliminary studies on the behavior of some enzymes in swine fever. Atti Soc. ital. Sci. vet. 20: 758-760.

Compagnucci, M. and Martone, F. 1967. Biochemical studies on swine fever. Activity of some serum enzymes. Acta Med. Vet. 13: 267-302.

Coons, A. H., Creech, H. J., Jones, R. N. and Berliner, E. 1942. The demonstration of pneumococcal antigen in tissues by the use of fluorescent antibody. J. Immunol. 45: 159-170.

Cornelius, C. E. 1960. Serum transaminases in veterinary diagnostics. Calif. Vet. 13, No. 6: 22-23.

Cornelius, C. E. 1963. Relation of body-weight to hepatic glutamic pyruvic transaminase activity. Nature 200: 580-581.

Cornelius, C. E., Bishop, J., Switzer, J. and Rhode, E. A. 1959. Serum and tissue transaminase activities in domestic animals. Cornell Vet. 49: 116-126.

Danilevskii, V. M. 1960. Electrophoretic protein pattern of the serum of pigs. Veterinariya 37: 41-43. Original not available; abstracted in Vet. Bull. 30: 656.

Dawson, J. B. 1960. The E.S.R. in a new dress. Brit. Med. J., June, 1960: 1697-1704.

DeSchweinitz, E. A. and Dorset, M. 1903. A form of hog cholera not caused by the hog cholera bacillus. U.S. Dept. Agr. Circular 41.

Deutsch, H. F. and Goodloe, M. B. 1945. An electrophoretic survey of various animal plasmas. J. Biol. Chem. 161: 1-20.

Dinwiddie, R. R. 1914. Studies on the hematology of normal and cholera infected hogs. Arkansas Agr. Expt. Sta. Tech. Bull. 120.

Dougherty, R. W., Shuman, R. D., Mullenax, C. H., Witzel, D. A., Buck, W. B., Wood, R. L. and Cook, H. M. 1965. Physiopathological studies of erysipelas in pigs. Cornell Vet. 55: 87-109.

Dressler, J. and Mach, P. 1967. Development of the activity of intracellular enzyme systems of piglet blood from birth to weaning. II. Alkaline phosphomonoesterase of neutrophils. Zentr. Veterinärmed. 14A: 548-553. Original not available; abstracted in Vet. Bull. 38: 183-184.

Dukes, H. H. 1955. The physiology of domestic animals. 7th ed. Ithaca, N.Y., Comstock Publishing Assoc.

Dunne, H. W. 1963. Field and laboratory diagnosis of hog cholera. Vet. Med. 58: 222-239.

Dunne, H. W. 1964a. Hog cholera. In Dunne, H. W., ed. Diseases of swine. 2nd ed. Pp. 140-186. Ames, Iowa, Iowa State University Press.

Dunne, H. W. 1964b. Hog cholera diagnostic problems. Modern Vet. Practice 45, No. 7: 76.

Dunne, H. W., Luedke, A. J. and Hokanson, J. F. 1958. The growth of animal leukocytes and their use in the cultivation of animal viruses. Am. J. Vet. Research 19: 706-711.

Enzyme nomenclature. Recommendations 1964 of the International Union of Biochemistry. 1965. New York, N.Y., American Elsevier Publishing Co.

Fujita, H. 1939. Uber die Microbestimmung der Blut Phosphatase. J. Biochem. 30: 69.

Gambino, S. R., Dire, J. J., Montelsone, M. and Budd, D. C. 1965. The Westergren sedimentation rate using K_3EDTA. Tech. Bull. Registry Med. Technol. 35: 173-180.

Gibson, J. P., Griesemer, R. A. and Koestner, A. 1965. Experimental distemper in the gnotobiotic dog. Pathol. vet. 2: 1-19.

Goldman, M. 1954. Use of fluorescein-tagged antibody to identify cultures of Endamoeba histolytica and Endamoeba coli. Am. J. Hyg. 59: 318-325.

Gornall, A. G., Bardawill, C. J. and David, M. M. 1949. Determination of serum proteins by means of the biuret reaction. J. Biol. Chem. 177: 751-766.

Grunbaum, B. W., Zec. J. and Durrum, E. L. 1963. Application of an improved microelectrophoresis technique and immunoelectrophoresis of the serum proteins on cellulose acetate. Microchem. J. 7: 41-53.

Hansard, S. L. and Kincaid, E. 1956. Red cell life span of farm animals. J. Animal Sci. 15: 1300-1306.

Hanson, R. P. 1957. The origin of hog cholera. J. Am. Vet. Med. Assoc. 131: 211.

Horzinek, M., Reczko, E. and Petzoldt, K. 1967. On the morphology of hog cholera virus. Arch. ges. Virusforsch. 21: 475-478.

Huhn, R. G., Osweiler, G. D. and Switzer, W. P. 1969. Application of the orbital sinus bleeding technique to swine. Lab. Animal Care 19: 403-405.

Janda, J. and Pařízek, J. 1967. Distribution of alkaline phosphatase, acid phosphatase, and non-specific esterase in the normal mucous membrane of the pig's stomach. Vet. Med., Praha 12: 157-164. Original not available; abstracted in Vet. Bull. 38: 50.

Jensen, W. N., Bush, J. A., Ashenbrucker, H., Cartwright, G. E. and Wintrobe, M. M. 1956. The kinetics of iron metabolism in normal growing swine. J. Exp. Med. 103: 145-153.

Kaplan, A. and Savory, J. 1965. Evaluation of a cellulose-acetate electrophoresis system for serum protein fractionation. Clin. Chem. 11: 937-942.

Kernkamp, H. C. H. 1939. The blood picture in hog cholera. J. Am. Vet. Med. Assoc. 95: 525.

Kernkamp, H. C. H. 1961. The natural history of hog cholera. In Mainwaring, G. T. and Sorensen, D. K., eds. Symposium on hog cholera. Pp. 19-28. St. Paul, Minnesota, University of Minnesota.

King, W. E. and Wilson, R. H. 1910. Studies on hog cholera and preventive treatment. Kansas Agr. Expt. Sta. Bull. 171.

Klimov, N. M., Malakhov, A. G. and Isaenko, E. P. 1959. Purification of swine fever virus by electrophoresis. Tr. Vses. Inst. Eksperim. Vet. 22: 195-201. Original not available; abstracted in Vet. Bull. 30: 504.

Knill, L. M., Podleski, T. R., and Childs, W. A. 1958. Proc. Soc. Exp. Biol. Med. 97: 224-225.

Köhler, H. 1956. Knochenmark und Blutbild des Ferkels. II. Das Ferkel mit spontaner Anämie. Zentr. Veterinärmed. 3: 460.

Kolb, E. and Schütz, W. 1961. Acid and alkaline phosphatase activity in the mucosa of the gastro-intestinal tract of pigs. Arch. exp. Veterinärmed. 15: 1274-1281.

Konopatkin, A. A. 1962. Immunologic studies with live hog cholera vaccine. Tr. Vses. Inst. Eksperim. Vet. 29: 166-176. Original not available; abstracted in Smithcors, J. F. and Catcott, E. J. Progress in swine practice. Vol. 1. P. 150. Santa Barbara, California, American Veterinary Publications, Inc.

Konopatkin, A. A. 1969. Plasma-cell reaction and immunological criteria during single or combined vaccination against swine fever and erysipelas. Doklady Vsesoyuz. Akad. Sel'skokhoz. Nauk. No. 5: 36-37.

Kornegay, E. T. 1967. Daily and twice daily repeated blood sampling in weanling pigs. Am. J. Vet. Research 28: 839-844.

Kostyunina, V. F. and Bychkov, I. S. 1965. The proteins and free amino-acids in the blood serum of healthy pigs and pigs with swine fever. Tr. Mosk. Vet. Akad. 48: 83-88. Original not available; abstracted in Vet. Bull. 36: 731.

Kubin, G. and Kölbl, O. 1968. Suitability of the immunofluorescence technique applied to frozen sections for swine fever diagnosis. Wien. tierärztl. Monatsschr. 55: 578-586. Original not available; abstracted in Vet. Bull. 39: 341-342.

Kubin, G. and Kölbl, O. 1969. Lymph-node biopsy for the fluorescent serological diagnosis of classical swine fever. Zentr. Veterinärmed. 16B: 305-311.

Lahey, M. E., Gubler, C. J., Chase, M. S., Cartwright, G. E. and Wintrobe, M. M. 1952. Studies on copper metabolism. II. Hematologic manifestations of copper deficiency in swine. Blood 7: 1053-1074.

Lewis, L. L., Schuler, W. P., McElroy, C. H. and Ritter, L. B. 1914. Hog cholera. Oklahoma Agr. Expt. Sta. Bull. 104.

Lewis, P. A. and Shope, R. E. 1929a. The blood in hog cholera. J. Exp. Med. 50: 719-737.

Lewis, P. A. and Shope, R. E. 1929b. The study of the cells of the blood as an aid to diagnosis of hog cholera. J. Am. Vet. Med. Assoc. 74: 145-152.

Lin, T. C., Kang, B. J., Shimizu, Y., Kumagai, T. and Sasahara, J. 1969. Evaluation of the fluorescent antibody-cell culture test for detection and titration of hog cholera virus. Nat. Inst. of Animal Health Quart. 9, No. 1: 10-19.

Loan, R. W. and Gustafson, D. P. 1964. Persistent infections of subculturable swine buffy coat cells with hog cholera virus. Am. J. Vet. Research 25: 1120-1122.

Lolin, M. 1957. Blood picture in pigs infected with swine fever virus, following vaccination with crystal violet vaccine. Acta Vet. Belgrade 7: 73-80. Original not available; abstracted in Vet. Bull. 29: 13-14.

Long, C. H., Ullrey, D. E. and Miller, E. R. 1965. Serum alkaline phosphatase in the post-natal pig and effect of serum storage on enzyme activity. Proc. Soc. Exp. Biol. Med. 119: 412-414.

Lynch, M. J., Raphael, S. S., Mellor, L. D., Spare, P. D. and Inwood, M. J. H. 1969. Medical laboratory technology and clinical pathology. 2nd ed. Philadelphia, Pa., W. B. Saunders Co.

MacGregor, R. G. S., Richards, W. and Loh, G. L. 1940. The differential leukocyte count. J. Pathol. Bacteriol. 51: 337.

Maciak, T. 1969. The effect of "Rovac" lapinized swine fever virus on animals infected or immunized with swine erysipelas bacteria. Arch. exp. Veterinärmed. 23: 201-226. Original not available; abstracted in Vet. Bull. 39: 780.

Mathews, J. and Buthala, D. A. 1955. Electrophoretic determination of the immunological response of swine to hog cholera. Vet. Med. 50: 213-216.

Mathews, J. and Buthala, D. A. 1956. Effect of repeated freezing and thawing on the electrophoretic pattern of swine serum. Am. J. Vet. Research 17: 485-486.

Mathews, J. and Buthala, D. A. 1960. Hog cholera protection tests with swine serum fractions. II. Hog cholera immune and non-immune serums. Cornell Vet. 50: 177-182.

Matthaeus, W. and Korn, G. 1969. Pathogenesis of swine fever from the time of infection until maximal pyrexia, and observations on serum macroglobulin. Zentr. Veterinärmed. 16B: 312-326. Original not available; abstracted in Vet. Bull. 39: 779-780.

Mayer, H. 1965. Der Einflussvon körperlicher Belastung auf die Aldolase-Kreatin phosphokinase - und Glutamat-Oxalacetat-Transaminase-Aktivitäten in Serum von Schlachtschweinen. Unpublished Ph.D. thesis. Giessen, Germany, Medical and Legal Veterinary Clinic, University of Giessen.

Mayr, A., Bachmann, P. A., Sheffy, B. E. and Siegl, G. 1967. Electron optical and buoyant density studies of hog cholera virus. Arch. ges. Virusforsch. 21: 113-119.

Mengeling, W. L. and Cheville, N. F. 1968. Host response to persistent infection with hog cholera virus. U.S. Livestock Sanitary Assoc. Proc. 72: 283-296.

Mengeling, W. L. and Drake, L. 1969. Replication of hog cholera virus in cell culture. Am. J. Vet. Research 30: 1819-1824.

Mengeling, W. L., Gutekunst, D. E., Fernelius, A. L. and Pirtle, E. C. 1963a. Demonstration of an antigenic relationship between hog cholera and bovine viral diarrhea viruses by immunofluorescence. Can. J. Comp. Med. Vet. Sci. 27: 162-164.

Mengeling, W. L. and Packer, R. A. 1969. Pathogenesis of hog cholera: host response. Am. J. Vet. Research 30: 409-417.

Mengeling, W. L., Pirtle, E. C. and Tarrey, J. P. 1963b. Identification of hog cholera viral antigen by immunofluorescence. Application as a diagnostic and assay method. Can. J. Comp. Med. Vet. Sci. 27: 249-252.

Mihajlovic, B., Jermolenko, G. and Kuzmanovic, M. 1969. Immunofluorescent investigations on the swine fever virus. Zentr. Bakteriol. Parasitenk. Abt. I. Orig. 209: 300-305.

Mikhailov, P. N., Kondrakhina, K. N. and Smutin, V. A. 1965. Protein and protein fractions in the blood of sows. Doklady Vsesoyuz. Akad. Sel'skokhoz. Nauk 7: 30-33. Original not available; abstracted in Vet. Bull. 36: 453.

Miller, E. R., Ullrey, D. E., Ackerman, I., Schmidt, D. A., Hoefer, J. A. and Luecke, R. W. 1961. Swine hematology from birth to maturity. I. Serum proteins. J. Animal Sci. 20: 31-35.

Møller, J. O. and Westergaard, U. 1964. Acid phosphatase in pig serum. Aarsberetn. Inst. Sterilitetsforsk. 1964: 139-146. Original not available; abstracted in Vet. Bull. 35: 118.

Moore, D. H. 1945. Species differences in serum protein patterns. J. Biol. Chem. 161: 21-32.

Mulhern, F. J. 1961. Why hog cholera eradication. In Mainwaring, G. T. and Sorensen, D. K., eds. Symposium on hog cholera. Pp. 13-18. St. Paul, Minnesota, University of Minnesota.

Nafstad, H. J. and Nafstad, I. 1968. An electron microscopic study of normal blood and bone marrow in pigs. Pathol. vet. 5: 451-470.

Nafstad, I. 1965. Studies of hematology and bone marrow morphology in vitamin E-deficient pigs. Pathol. vet. 2: 277-287.

Nafstad, I. and Nafstad, H. J. 1968. An electron microscopic study of blood and bone marrow in vitamin E-deficient pigs. Pathol. vet. 5: 520-537.

National Academy of Sciences. 1968. Nutrient requirements of swine. 6th ed. Washington, D.C., National Academy of Sciences.

Noyan, A. 1949. A study of bone marrow in farm animals. Doctoral Diss. 60: 237.

Orstadius, K., Wretlind, B., Lindberg, P., Nordstrom, G. and Lannek, N. 1959. Plasma-transaminase and transferase activities in pigs affected with muscular and liver dystrophy. Zentr. Veterinärmed. 6: 971-980.

Patt, H. M. and Maloney, M. A. 1959. Patterns of neutrophilic leukocyte development and distribution. Ann. New York Acad. Sci. 77: 766-775.

Pehl, K. H. and Schulze, W. 1958. Der Virusgehalt blutfreier Organe bei der Schweinepest. Arch. exp. Veterinärmed. 12: 861-869. Original not available; abstracted in Vet. Bull. 29: 437.

Pirtle, E. C. 1969a. In vitro spread of hog cholera viral infection from cell to cell: Demonstration of viral antigen in cytoplasmic bridges. J. Am. Vet. Med. Assoc. 30: 1913-1919.

Pirtle, E. C. 1969b. In vitro spread of hog cholera viral infection from cell to cell: Demonstration of viral antigen in dividing cells. J. Am. Vet. Med. Assoc. 30: 1909-1912.

Poul, J. 1962. Serum proteins in pigs during hyperimmunization against swine fever. Arch. Inst. Pasteur Algerie 40: 44-52. Original not available; abstracted in Vet. Bull. 33: 25.

Quin, A. H. 1950. The past and future of hog cholera control. J. Am. Vet. Med. Assoc. 116: 411.

Regner, A. 1923. Ein Beitrag Zum Blutbilde des gesunden und Kranken Schweines und dessen Verwertung bei Diagnose von Rotlauf, Schweinepest und Schweineseuche. Wien. tierärztl. Monatsschr. 10: 97.

Reitman, S. and Frankel, S. 1957. Colorimetric method for the determination of serum transaminase activity. Am. J. Clin. Path. 28: 56-63.

Report of the committee for the clarification of the nomenclature of cells and diseases of the blood and blood forming organs. 1949. Blood 4: 89.

Ressang, A. A. and DeBoer, J. L. 1969. The indirect fluorescent antibody technique as a method for detecting serum antibodies against hog cholera. I. An outline of the technique and its preliminary evaluation. Zentr. Veterinärmed. 16: 709-716.

Ritchie, A. E. and Fernelius, A. L. 1967. Electron microscopy of hog cholera virus and its antigen-antibody complex. Vet. Record 81: 417-418.

Ritchie, A. E. and Fernelius, A. L. 1968. Direct immunoelectron microscopy and some morphological features of hog cholera virus. Arch. ges. Virusforsch. 23: 292-298.

Rohovsky, M. W. and Griesemer, R. A. 1967. Experimental feline infectious enteritis in the germfree cat. Pathol. Vet. 4: 391-410.

Roszkowski, J. 1967. Histochemical investigations on alkaline phosphatase activity in the adrenal cortex and intestine of swine infected with the American strain of hog cholera virus. Bull. Vet. Inst. Pulawy 11: 108-113. Original not available; abstracted in Vet. Bull. 37: 487.

Sainte-Marie, G. 1962. A paraffin embedding technique for studies employing immunofluorescence. J. Histochem. Cytochem. 10: 250-256.

Salmon, D. E. and Smith, T. 1886. Investigations in swine plague. Bureau of Animal Industry Annual Report 2: 184-246.

Schalm, O. W. 1965. Veterinary hematology. 2nd ed. Philadelphia, Pa., Lea and Febiger.

Sippel, W. L. 1952. White blood cell count in hog cholera. Vet. Med. 47: 497-501.

Sippel, W. L. 1958. Practitioners laboratory. Vet. Med. 53: 341-342.

Sirbu, Z., Bran, L. and Michailov, L. 1968. Identification of lapinized swine fever virus by the fluorescent antibody technique. Arhiva vet. 4: 19-25. Original not available; abstracted in Vet. Bull. 39: 417-418.

Sirbu, Z., Ieremia, D. and Bona, C. 1964. Immunofluorescent microscopy in the diagnosis of swine fever. Brit. Vet. J. 120: 587-591.

Šlesingr, L. 1964. Activity of SGOT, SGPT, aldolase and dehydrogenase of sorbitol and maleic and lactic acid, in sheep and pig serum. Monatsh. Veterinaerm. 19: 663-665. Original not available; abstracted in Vet. Bull. 35: 118.

Šlesingr, L. 1965. Enzyme activity of SGOT, SGPT, aldolase, leucyl aminopeptidase, lactic and malic dehydrogenase in influenza-bronchopneumonia of pigs. Monatsh. Veterinaerm. 20: 573-574. Original not available; abstracted in Vet. Bull. 36: 46.

Solorzano, R. F. 1962. A fluorescent antibody test for hog cholera. Unpublished Ph.D. thesis. University Park, Pennsylvania, Library, Pennsylvania State University.

Solorzano, R. F., Thigpen, J. E., Bedell, D. M. and Schwartz, W. L. 1966. The diagnosis of hog cholera by a fluorescent antibody test. J. Am. Vet. Med. Assoc. 149: 31-34.

Sorensen, D. K., Martinsons, E. and Perman, V. 1961. Clinical and hematological manifestations of hog cholera In Mainwaring, G. T. and Sorensen, D. K., eds. Symposium on hog cholera. Pp. 29-42. St. Paul, Minnesota, University of Minnesota.

Stair, E. L., Rhodes, M. B., Aiken, J. M., Underdahl, N. R. and Young, G. A. 1963. A hog cholera virus-fluorescent antibody system. Its potential use in study of embryonic infection. Proc. Soc. Exp. Biol. Med. 113: 656-660.

Stair, E. L., Rhodes, M. B., Grace, O. D. and Aiken, J. M. 1964. Fluorescent antibody for diagnosis of hog cholera. U.S. Livestock Sanitary Assoc. Proc. 67: 599-606.

Teebken, D. L., Aiken, J. M. and Twiehaus, M. J. 1967. Differentiation of virulent, attenuated and inactivated hog cholera viruses by fluorescent-antibody technique. J. Am. Vet. Med. Assoc. 150: 53-58.

Turubatović, R. and Panjević, D. 1957. Proteinemia in pigs immunized and nonimmunized against swine fever. Acta Vet. Belgrade 7: 61-71. Originial not available; abstracted in Vet. Bull. 29: 14.

U.S. Dept. Agr. Bureau of Animal Industry. 1888. The introduction and spread of hog cholera in the United States. Fourth and fifth annual reports. Washington, D.C., U.S. Govt. Print. Off.

Varicák, D. 1935. Zur Kenntnis des Markes der Rumpfknochen. Untersuchungen zwecks klinischer Auswertung und Pferd, Rind, Schwein, Hunde und Katze. Arch. wiss. u. prakt. Tierheilk. 73: 461.

Vesselinovitch, S. D. 1955. Electrophoretic studies of oedema disease in swine. Brit. Vet. J. 111: 398-403.

Watanabe, Y., Watanabe, K. and Hinuma, Y. 1962. Synthesis of poliovirus specific proteins in HeLa cells. Biochem. Biophys. Acta 61: 976-977.

Weber, W. T. 1969. Evaluation of bone marrow. In Medway, W., Prier, J. E. and Wilkinson, J. S., eds. A textbook of veterinary clinical pathology. Pp. 299-315. Baltimore, Maryland, Williams and Wilkins Co.

Wegger, I. and Westergaard, U. 1963. Glutamic oxaloacetic transaminase and alkaline phosphatase in pig serum. Aarsberetn. Inst. Sterilitetsforsk. 1963: 115-126. Original not available; abstracted in Vet. Bull. 34: 228.

Weide, K. D. and King, N. B. 1962. Electrophoretic studies of passive immunity against hog cholera in young pigs. Am. J. Vet. Research 23: 744-747.

Weide, K. D., Sanger, V. L. and Lágacé, A. 1962. Inoculation of baby pigs with lapinized hog cholera vaccine. J. Am. Vet. Med. Assoc. 141: 464-475.

Weide, K. D. and Twiehaus, M. J. 1959. Hematological studies of normal, ascarid infected and hog cholera-vaccinated swine. Am. J. Vet. Research 20: 562-567.

Wintrobe, M. M. 1967. Clinical hematology. 6th ed. Philadelphia, Pa., Lea and Febiger.

Wintrobe, M. M. and Landsberg, J. W. 1935. A standardized technique for the blood sedimentation test. Am. J. Med. Sci. 189: 102.

Wisecup, W. G. and Crouch, B. G. 1963. Evaluation and calibration of an electronic particle counter for enumeration of multispecies blood cells. Am. J. Clin. Pathol. 39: 349-354.

Witzel, D. A., Wood, R. L. and Buck, W. B. 1967. Changes in plasma glucose level and SGOT activity in acute swine erysipelas and inappetence in pigs. Cornell Vet. 57: 70-78.

Wretlind, B., Orstadius, K. and Lindberg, P. 1959. Transaminase and transferase activities in blood plasma and in tissues of normal pigs. Zentr. Veterinärmed. 6: 963-970.

ACKNOWLEDGEMENTS

The counsel, guidance and encouragement of Dr. J. R. Duncan, my major professor, are gratefully acknowledged.

Special thanks are due to Dr. F. K. Ramsey for his interest and support during this study.

The author also wishes to acknowledge the interest, time and advice of Dr. M. W. Sloss, Dr. R. A. Packer, Dr. R. C. Ewan and Dr. H. A. McDaniel who are the other members of the graduate committee.

This dissertation is affectionately dedicated to my wife, Sonya, and daughter, Holly, who shared an existence in place of a life with me during the past six years.

A special note of appreciation to Mrs. P. A. Clark and Mrs. K. L. Tomsom for their expert technical work and editorial assistance.

A note of gratitude to Mr. C. K. Graham and Mr. Gordon Booth for their excellent guidance with the biometry utilized in this study.

Thanks are given to Drs. W. C. Stewart and H. A. Nelson, Mr. W. E. Minert, Mr. J. A. Fitzgerald and J. E. Beattie for their technical assistance.

Photographic assistance by Mr. Ralph Glazier, Mr. Wayne Romp and Mr. Tom Glasson is also acknowledged.

The cooperation of Mrs. S. Hulse and P. Heiser in the typing of this manuscript is greatly appreciated.